A Pilot Clinical Trial Assessing the Safety and Feasibility of Intramuscular Administration of the TA-CIN Vaccine as Adjuvant Therapy for Patients with History of HPV16 Associated Cervical Cancer

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A Pilot Clinical Trial Assessing the Safety and Feasibility of Intramuscular Administration of the TA-CIN Vaccine as Adjuvant Therapy for Patients with History of HPV16 Associated Cervical Cancer
- PROTOCOL REVISION RECORD -

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April 20, 2015 (Version 1.1)

Revisions:

Amendment #1: February 7, 2016
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Amendment # 2: November 16, 2016
Sections Revised: *Title, Schema, Section 1, Section 2.3, Section 3.6, Section 4.1, Section 6, Section 9, Section 10, Section 12, Appendix D*

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Sections Revised: *All to reflect change in dose*

Amendment #4: February 7, 2019
Sections Revised:

Amendment #5: October 4, 2019
Sections Revised: *Schema, Section 10 (Footnote #5), Appendix C, Exclusion Criteria*

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Sections Revised: *Change in PI (1st page, header, Appendix C)*

Amendment #5.2: June 10, 2020
Sections Revised: *DSMB*

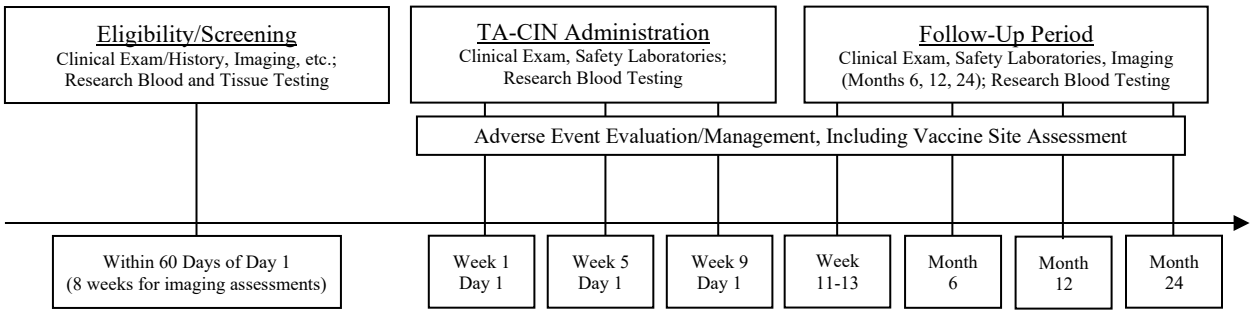
Amendment #5.3: July 26, 2020
Sections Revised:

Amendment #6: October 21, 2022
Sections Revised: *Change in Statistician, first page, enrollment total updated to 15*

SCHEMA

Eligible:
Women With HPV16-Associated Stage IB1-IV Cervical Cancer;
Completed Definitive Treatment Within 12 Months.

Study Design:



Treatment Groups/Cohorts					
Group	TA-CIN Route of Administration	Volume (mL)	Dose (µg)	Treatment Frequency/Weeks	# of Patients
1	Intramuscular Arm	0.53	100	3 administrations per subject; Weeks 1, 5, and 9	7
2	Intramuscular Thigh				7

Table of Contents

1. SUMMARY

This is a randomized, multi-center, open label pilot study. The primary goal of this study is to determine the safety of TA-CIN vaccine as adjuvant therapy, and to assess evidence of induction of HPV antigen-specific immunologic response when administered at different locations. TA-CIN has proven to be well-tolerated in healthy humans when administered three times monthly intramuscularly in the deltoid at a 533µg dose. In this pilot study, a single dose level (100µg) assessment of the safety and tolerability of administering TA-CIN vaccine three times to either the arm versus the thigh of patients who have previously been treated for HPV16-related cervical cancer in the past year and are documented to have no evidence of disease recurrence based on standard-of-care imaging and/or clinical assessment upon eligibility. Since TA-CIN administration is likely to have low toxicity, an important element of this study is to analyze immunologic parameters to examine whether one of the two immunization sites induces greater HPV-specific immunogenicity. The immunogenicity analyses include: 1) assessment of T cell-mediated immune responses of peripheral blood lymphocytes to HPV16 E7E6 stimulation by ELISPOT, and if not discriminatory then 2) by T cell proliferation assays, or 3) by measurement of HPV16 L2E7E6-specific antibodies by ELISA (**Section 10)**,. These analyses will be combined with toxicity data to select a vaccination site for a future clinical trial(s).

A total of 15 patients will be enrolled to assess the safety of TA-CIN vaccine via different injection sites as adjuvant therapy. Patients with HPV16-associated stage IB1-IV cervical cancer who completed definitive cancer treatment within 1 year before the initiation of vaccination and are documented to have no evidence of disease recurrence based on standard-of-care imaging and/or clinical assessment will be eligible. This population was chosen because women with HPV-associated cervical cancer may potentially benefit from the vaccination strategy as adjuvant therapy as compared to healthy volunteers with respect to prevention or delay in the development of HPV16 associated cervical cancer recurrence. One dose level of TA-CIN (100µg) delivered via one of two different intramuscular injection sites is planned (**Section 6**).

Safety assessments will continue for a period for 1 month after the last vaccination; reporting requirements, exclusions, and stopping rules are outlined (**Sections 7 and 8**). Subjects who withdraw from the study for reasons other than toxicity may be replaced at the discretion of the Principal Investigator and/or IND sponsor.

Few or no serious adverse events (SAEs) are expected from this regimen and routes of administration. The motivation for the design is to confirm that the dose and site of injection implemented here has minimal or no systemic toxicity, as well as determining the preferred injection site that can elicit more potent immune response.

Patient immune and clinical responses in cohorts with different injection sites will be evaluated through the following methods: 1) peripheral blood lymphocytes (PBL) to assess HPV16 E6 and E7-specific $CD8^+$ T cell ELISPOT and lymphocyte proliferative responses to HPV16 antigenic stimulation and T cell receptor sequencing; 2) serum for HPV16 L2, E6, and E7 and neutralizing antibody responses; 3) plasma for residual HPV viral load; and, 4) imaging scans per standard-of-care (i.e., every 6-12 months) and as clinically indicated to assess time to disease recurrence.

2. OBJECTIVES

2.1 Primary Objectives

2.1.1 To determine the safety and feasibility of intramuscular administration of TA-CIN vaccine via arm or thigh in patients with a history of HPV16 associated IB1-IV cervical cancer

2.2 Secondary Objectives

2.2.1 To evaluate the levels of circulating antibody to HPV16 E6, E7, and L2 in the peripheral blood pre- and post-vaccination by ELISA

2.2.2 To evaluate the levels of circulating HPV16 E6- and E7- specific $CD8^+$ T cells and/or CD4+ T cells in the peripheral blood pre- and post-vaccination using the ELISPOT assay and/or cell surface staining with multi-parameter flow cytometry analysis

2.2.3 To evaluate the proliferative responses of peripheral blood mononucleocytes pre- and post-vaccination in response to stimulation by HPV16 E6, E7 and L2

2.3 Exploratory Objective(s)

2.3.1 To evaluate the levels of circulating HPV16 E6- and E7-specific $CD8^+$ T cells in the peripheral blood pre- and post-vaccination using T cell receptor sequencing

2.3.2 To evaluate the levels of HPV-specific neutralizing antibodies in the peripheral blood pre- and post-vaccination

2.3.3 To assess residual HPV16 viral load in plasma

2.3.4 To assess time to disease recurrence as a measure of clinical response that may be associated with vaccine-induced immune responses

3. BACKGROUND

3.1 Cervical Cancer

Globally, cervical cancer accounted for an estimated 530,000 new cancer cases worldwide and for 275,000 deaths in 2013 (*1*). The most common histologic types of cervical cancer are squamous cell (69 percent of cervical cancers) and adenocarcinoma (25 percent). In countries that do not have access to cervical cancer screening and prevention programs, cervical cancer remains the second most common type of cancer and cause of cancer deaths among all types of cancer in women (*2*). In the United States (U.S.), an estimated 12,000 women diagnosed with cervical cancer each year (*3*). It is less common in the U.S. because of the routine use of Pap smear testing in intervention via ablation on high grade cervical intraepithelial neoplasia (CIN). Prior to the introduction of the Pap test in the 1940s, cervical cancer was one of the leading causes of

cancer death for women in the United States. But after routine cervical cancer screening was initiated, cervical cancer incidence was reduced by 70 percent and deaths from cervical cancer by 90 percent. Despite treatment advances over the past several decades including radiotherapy, cisplatin-based chemotherapy and/or surgery, the overall 5-year survival in the U.S. has not changed significantly and remains approximately 30% (http://www.cancer.org/Cancer/CervicalCancer/DetailedGuide/cervical-cancer-survival). The human papillomavirus (HPV) is central to the development of cervical dysplasia and cancer is detected in 99.7 percent of cervical cancers.

3.2 Human Papillomavirus as an Etiologic Factor in a Distinct Subset of Cervical Cancers

Human papillomaviruses (HPV) are small, non-enveloped DNA viruses that induce self-limited epithelial lesions of the skin or mucosa. Of the more than 150 genotypes of HPV that have been identified, a dozen have been found to induce lesions that may progress to cancer. Persistent infection with one of about 14 genotypes of carcinogenic human papillomavirus (HPV) causes almost all cases, and 70% of cervical cancer cases in the U.S. are associated with HPV 16 or 18 (*4*). The International Agency for Research on Cancer (IARC) concluded that there is sufficient evidence in humans to establish the carcinogenicity of HPV in causing cervical cancer and a subset of head and neck cancers. HPV DNA has been detected in the tumor nuclei of up to approximately 99% of all cervical cancers. HPV type 16 (HPV16) is the most prevalent HPV type detected in cervical cancers, involved in approximately 50-60%. Given the causative role of HPV16 in at least half of cervical cancer cases, prophylactic vaccines targeting HPV16 have been developed for the prevention of HPV-related cervical malignancies. Unfortunately, the licensed preventive HPV vaccines (Gardasil® and Cervarix®) have no documented therapeutic activity for the treatment of cervical cancer. Therefore, there is an urgent need for new targeted treatments for cervical cancer to complement existing modalities such as radiation and/or cisplatin chemotherapy.

3.3 Importance of Cell-Mediated Immune Responses in Controlling HPV-Associated Tumors

Several lines of evidence highlight the importance of cell-mediated immune responses in controlling both HPV infections and HPV-associated neoplasms (for review, see (*5*)). First, the prevalence of HPV-related diseases (infections and neoplasms) is increased in transplant recipients on immunosuppressive therapies (*6*) and HIV-infected patients (*7, 8*), both of whom are known to have impaired cell-mediated immunity. HIV-infected women with baseline CD4+ T-cells of ≥350, 200-349, and <200 cells per microliter had a 2.3, 3.0, and 7.7 times increase in invasive cervical cancer incidence, respectively, compared with HIV-uninfected women (*9*). HPV16 associated oropharyngeal cancer has been reported in the post-transplant setting (*10*) and the incidence of HPV16 associated tonsillar cancer in HIV infected men has been reported to be 2.6 fold greater (*11*) than in non-HIV infected individuals. In addition, when patients on immunosuppressive therapies discontinue their treatment, their HPV-associated lesions regress (for review, see (*12*)). Infiltrating $CD4^+$ (T helper cells) and $CD8^+$ (cytotoxic T cells) T cells have been observed in spontaneously regressing HPV-associated lesions (*13*). Lastly,

preclinical studies have demonstrated that vaccinated animals are protected from papillomavirus infection and from the development of neoplasms, and vaccination also facilitates the regression of existing lesions (*14-16*). The link between HPV16-associated lesions and/or carcinoma and cell-mediated immunity presents the possibility of eradicating these lesions or cancers through vaccines which enhance HPV-specific T cell immune responses.

3.4 HPV Oncogenic Proteins, E6 and E7, as Potential Targets for Immunotherapy Against HPV-Associated Cervical Cancer

In the cellular progression from viral infection to malignant transformation, the episomal viral DNA can integrate into the host genome with a resultant deletion of non-critical viral genes. Expression of late genes (L1 and L2) and some early genes (E1 and E2) is typically lost, and E6 and E7 are often the only open reading frames consistently expressed in cancer cell lines (*17*) and in HPV-associated cancers (*18*), although they are expressed in productive precursor lesions. E6 and E7 are necessary for viral transformation through their inactivation of two human tumor suppressor proteins, p53 and pRb, respectively, resulting in dysregulated cell cycle proliferation, delayed cellular differentiation, increased frequency of spontaneous and carcinogenic-induced mutations, and increased chromosomal instability (*19*).

Since E6 and E7 are constitutively expressed in most HPV-associated cancers, they represent promising targets for the development of antigen-specific therapeutic vaccines. There are several advantages to targeting the E6 and/or E7 proteins. They are foreign viral proteins, and, therefore, are more immunogenic than a self-protein that is genetically altered (such as a mutated p53) or aberrantly reactivated (such as the embryonic protein, MAGE-1). Furthermore, since E6 and E7 are required for the induction and maintenance of the malignant phenotype of cancer cells (*20*), tumor cells cannot evade a directed immune response through antigen loss of E6 or E7. Lastly, preclinical studies suggest that vaccines targeting these early papillomavirus proteins can generate therapeutic as well as protective effects *in vivo* (*21*). Therefore, the E6 and E7 proteins represent logical targets for developing antigen-specific immunotherapies or vaccines directed against HPV-associated cervical cancers. Various forms of vaccines, such as vector-based vaccines, tumor-based vaccines, DNA-based vaccines and protein/peptide-based vaccines, have been described in experimental systems targeting the HPV16 E6 and/or E7 proteins (*22-32*).

3.5 Therapeutic Effects of the Standard Care of Chemoradiation for Cervical Cancer May be Improved by Therapeutic HPV Vaccines

The current standard of care for advanced HPV-associated cervical cancer includes the use of a chemotherapeutic drug, cisplatin, in conjunction with local radiation therapy. Despite improvements noted with combination therapy, five-year survival in most patients affected by advanced cervical cancer is approximately 30% (*33*). Thus, an innovative treatment strategy, via a distinct mechanism, that can improve outcomes in patients with advanced cervical cancer is needed. We have previously demonstrated that administration of a therapeutic HPV vaccine following chemotherapy with cisplatin or

radiation was able to improve the therapeutic antitumor effect in a preclinical model compared to vaccination, chemotherapy or chemoradiation alone (*34-36*). These data suggest that a therapeutic HPV vaccine may have value in improving the therapeutic effect of the standard care of chemoradiation for cervical cancer.

3.6 Safety of TA-CIN Vaccine

TA-CIN is a single fusion protein comprised of HPV16 E6, E7 and L2 proteins linked in tandem. Vaccination with TA-CIN induces immunity to HPV16 E6/E7-specific T cell mediated immune responses and L2-specific neutralizing antibodies in mice. Importantly, vaccination of HPV16 infected-patients with TA-CIN is also designed to trigger therapeutic immunity targeting the E6, E7 and L2 of HPV16. A placebo-controlled, double-blinded phase I dose escalation study provided preliminary evidence that serial vaccination with up to 533μg of TA-CIN for a total of three doses in the absence of an adjuvant is safe, well-tolerated and immunogenic in healthy volunteers (*37*). However, vaccination of patients produced only low titers of L2-specific cross-neutralizing antibodies and weak E6/E7-specific interferon (IFN)-γ and proliferative T cell responses following a TA-CIN dose response (*37, 38*). In a phase II clinical study, 29 patients with predominantly HPV16+ high grade vaginal or vulval intraepithelial neoplasia (VAIN or VIN), were vaccinated with 533 μg of TA-CIN followed by a single dose boost with recombinant vaccinia virus expressing both HPV16 and HPV-18 E6 and E7 genes (TA-HPV) (2.5×10^5 pfu). Combination TA-CIN and TA-HPV vaccination failed to induce an improved rate of lesion regression as compared to earlier studies utilizing either TA-HPV alone or TA-CIN alone in 30 VIN patients (*39*).

Another clinical trial tested TA-CIN vaccination following **treatment** with an immunomodulatory drug**, imiquimod**. Twenty women aged 18–70 years with biopsy-proven VIN grades 2 and 3 were recruited (19 evaluated) in a clinical trial examining combination treatment with topical imiquimod followed by 3 doses of TA-CIN vaccination (*40*). Exclusion criteria were pregnancy, invasive disease, immunosuppression, history of severe allergy and previous HPV vaccination. Imiquimod 5% cream was self-administered topically for 8 weeks, escalating from one application in week 1 to two in week 2 and three applications in weeks 3–8. This was followed by three intramuscular doses of TA-CIN (128 μg) at weeks 10, 14 and 18. The primary objective was to measure treatment effect on VIN by lesion size and histology and the secondary objectives were to assess lesion HPV status, symptoms, immune responses as well as safety, toxicity and tolerability. Women were reviewed at weeks 0, 10, 14, 18, 20, 26 and 52, the primary end point. Punch biopsies were taken for histology and HPV typing at weeks 0, 10, 20 and 52. Heparinized blood was obtained for immunological assays at weeks 0, 10 and 20. TA-CIN, which was administered intramuscularly into the deltoid muscle, was well tolerated with no side effects or adverse events (*40*).

The vaccination strategy of boosting with TA-CIN recombinant protein following priming with a different therapeutic HPV vaccine was investigated in a phase I clinical trial (*41*). 10 women with HPV16+ high grade VIN were primed with TA-HPV vaccinia vaccine followed by boosting with TA-CIN (533 μg/0.5 mL) three times at 1 month

intervals 7-15 months after TA-HPV priming vaccination. The results showed that the prime-boost vaccination strategy elicited strong HPV16-specific proliferative T cell and serological responses. Furthermore, lesion shrinkage or symptom relief was observed in 3 patients. Importantly, vaccination with TA-CIN was shown to be safe and well tolerated with no reported adverse events. In sum, the four clinical trials involving the use of TA-CIN in over 90 patients provide strong support for the safety and feasibility of three monthly intramuscular TA-CIN vaccinations at doses as high as 533µg.

3.7 Rationale for the Clinical Trial Design

3.7.1 TA-CIN is highly effective in tumor bearing mice but not in healthy mice

In a recent pre-clinical study, we have compared the HPV16-specific CD8 T cell immune responses elicited by three vaccinations with TA-CIN in the naïve or tumor bearing mice.

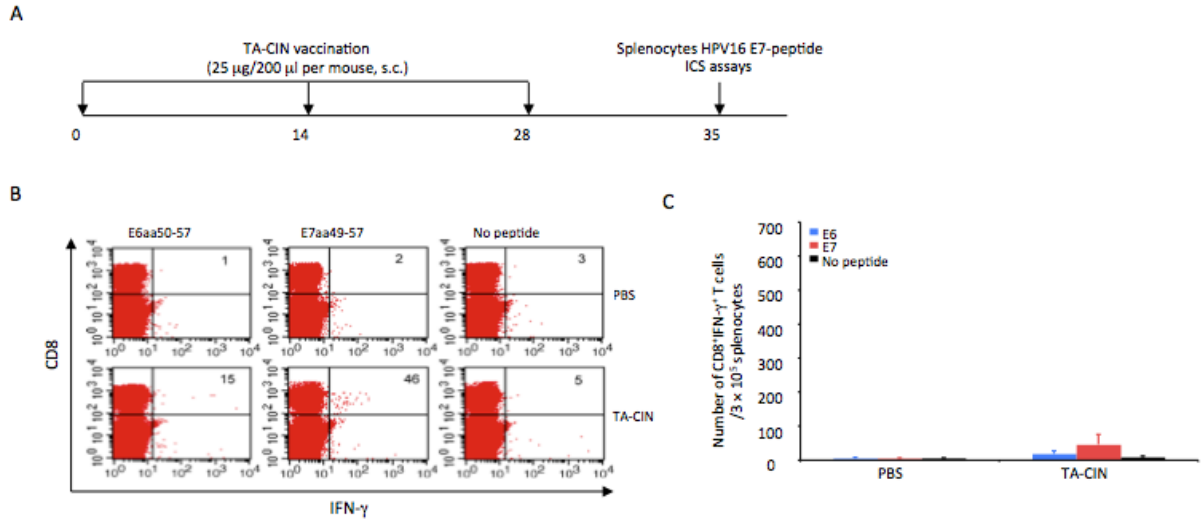


Figure 1. TA-CIN is not immunogenic in healthy mice. **A.** Schematic illustration of the experiment. Briefly, 5~8 weeks old female C57BL/6 mice (5 mice/group) were injected with vaccinated with either 25 µg/mouse of TA-CIN or PBS on day 0. The mice were boosted twice with the same regimen in 2 week interval. On day 35, the mice were sacrificed and the spleens were harvested. **B.** Flow cytometry analysis of HPV16 E6 and E7-specific $CD8^+$ T cell responses analyzed by IFN-γ intracellular staining. Briefly, splenocytes were collected, stimulated with 1 µg/ml HPV16 E6aa50-57, or E7aa49-57 peptide at the presence of GolgiPlug (1µl/ml) overnight at 37^0C. The cells were then stained with anti-mouse CD8 followed by intracellular IFN-γ. The data were acquired with FACSCalibur and analyzed with CellQuest. **C.** Summary of the flow cytometry data.

As shown in Figure 1, vaccination three times with TA-CIN alone is poorly immunogenic in healthy naïve mice. However, as shown in Figure 2, administration of TA-CIN to mice bearing HPV16 E6 and E7 expressing TC-1 tumor generated remarkably higher HPV16-specific CD8+ T cell responses as compared to the untreated mice. This was also associated with a potent therapeutic effect against the TC-1 tumor that had metastasized to lung. These results suggest that TA-CIN alone can be highly immunogenic and effective when administered into subject with preexisting exposure to HPV16-associated tumor.

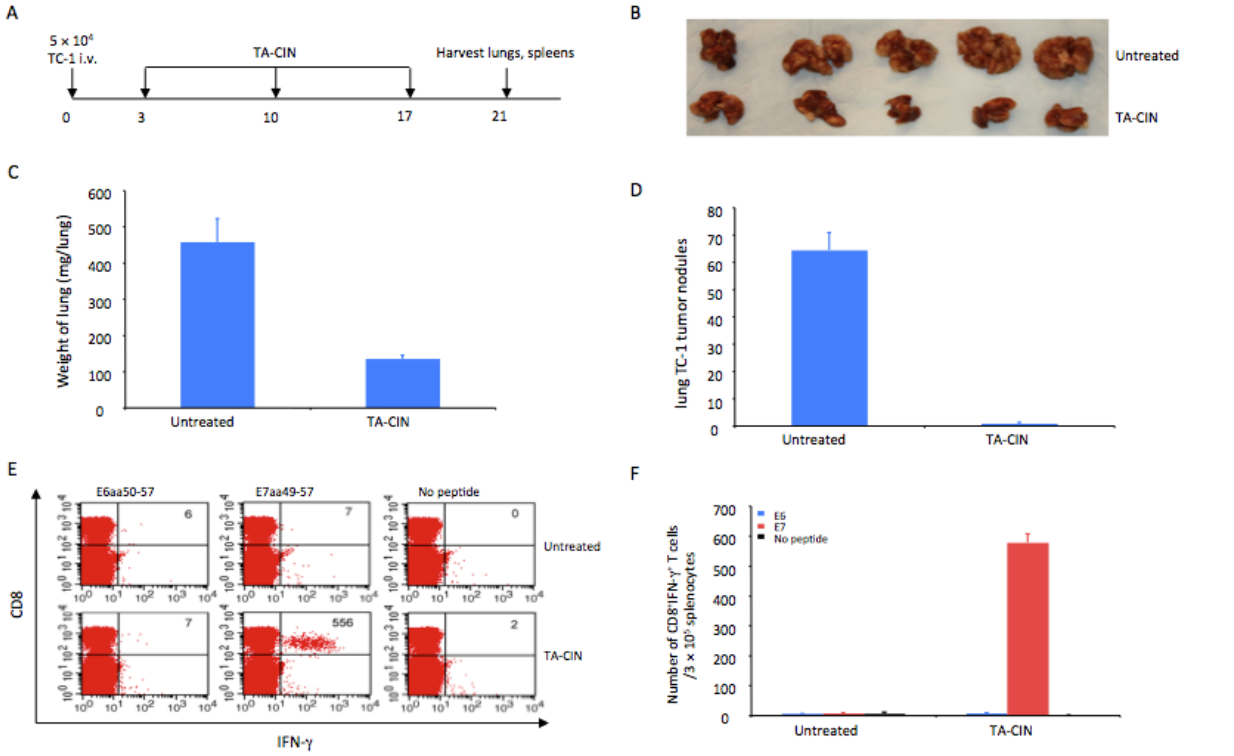


Figure 2. Therapeutic effect of TA-CIN against HPV16 E6E7-expressing TC-1 lung metastasis. **A.** Schematic illustration of the experiment. Briefly, 5~8 weeks old female C57BL/6 mice (5 mice/group) were injected with 5×10^4 TC-1 cells intravenously on day 0. On day 3, the mice were vaccinated with either 25 μg/mouse of TA-CIN via subcutaneous injection or left untreated. The mice were boosted twice with the same regimen. On day 21, the mice were sacrificed, lungs and spleens were harvested. The weight of the lungs was weighed, and the number of nodules of the tumor was counted. **B.** Images of lungs of the mice from the experiment. **C.** Summary of the weight of the lungs of the mice from the experiment. **D.** Summary of the number of TC-1 metastasis nodules. **E.** Flow cytometry analysis of HPV16 E6 and E7-specific $CD8^+$ T cell responses analyzed by IFN-γ intracellular staining. Briefly, splenocytes were collected, stimulated with 1 μg/ml HPV16 E6aa50-57, or E7aa49-57 peptide at the presence of GolgiPlug (1μl/ml) overnight at 37^0C. The cells were then stained with anti-mouse CD8 followed by intracellular IFN-γ. The data were acquired with FACSCalibur and analyzed with CellQuest. **F.** Summary of the flow cytometry data.

3.7.2 Site of Vaccination Affects the Anti-Tumor Immunity

In another pre-clinical study, we explored whether the site of vaccination will impact anti-tumor immunity, specifically whether intramuscular vaccination of the leg will generate better anti-tumor immunity than vaccination on the arm against cervical HPV disease. As a model for vaccination of cervical cancer patients in the arm versus the leg, we vaccinated mice bearing cervicovaginal, luciferase-expressing TC-1 tumor with a therapeutic HPV16 DNA vaccine pNGVL4A-CRT/E7(detox), administered either in the front leg (which drains to a lymph node distal to the cervical tumor) or the hind leg (which drains to the proximal lymph node shared with the cervical tumor).

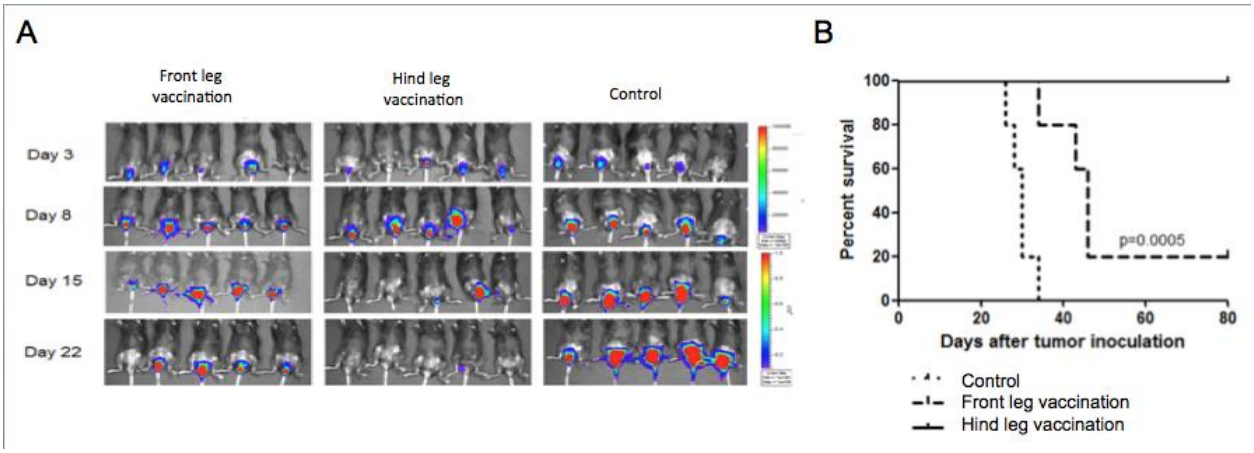


Figure 3. Characterization of therapeutic antitumor effect of therapeutic HPV vaccine administered at different site in cervicovaginal tumor model. 5~8 weeks old female C57BL/6 mice (5 mice/group) were injected with 5×10^4 of TC-1/ luciferase cells at the intravaginal cavity on day 0. The mice were vaccinated with 20 μg/mouse of pNGVL4a-CRT/E7 DNA via intramuscular injection follow by electroporation at different site on day 4 and boosted once 4 days later. **(A)** Bioluminescence images of the cervicovaginal TC-1/luciferase tumor-bearing mice. **(B)** Kaplan-Meier survival of the TC-1/luciferase tumor-bearing mice.

As shown in Figure 3, tumor bearing mice that received hind leg vaccination demonstrated a significantly greater reduction in luminescence signal as compared to the tumor bearing mice vaccinated at the front leg or tumor bearing mice with no vaccination, corresponding to a greater reduction in tumor volume. Furthermore, 100% of the tumor bearing mice with hind leg vaccination survived for at least 80 days while 80% of the tumor bearing mice with front leg vaccination and 100% of the tumor bearing mice with no vaccination died within 50 days after tumor challenge, demonstrating a significantly better anti-tumor effect against cervicovaginal tumor elicited by the therapeutic vaccination when administered at the hind leg of the tumor bearing mice.

To examine the impact of the site of vaccination upon antigen-specific immune response in mice bearing cervicovaginal tumor, we harvested the tumor and lymph nodes from the tumor bearing mice that received no vaccination, vaccination in front leg, or vaccination in hind leg 7 days after the last vaccination and analyzed each sample for E7-Specific CD8+ T cells. As shown in Figure 4 A-B, mice vaccinated at the hind leg generated significantly larger amount of E7-specific CD8+ tumor infiltrating lymphocyte as compared to mice vaccinated at the front leg.
When comparing the T cell populations in the lymph nodes, vaccination in the hind leg generated a significantly higher amount of E7-specific CD8+ T cells in the inguinal and iliac lymph node as compared to the axillary lymph node, while vaccination in the front leg generated comparable amount of E7-specific CD8+ T cells in the axillary, inguinal, and iliac lymph nodes (Figure 4 C-D). These results suggest that administration of a therapeutic HPV vaccine such as TA-CIN at a site adjacent to tumor-draining lymph nodes can enhance the generation of antigen-specific T cells and improve treatment outcome.

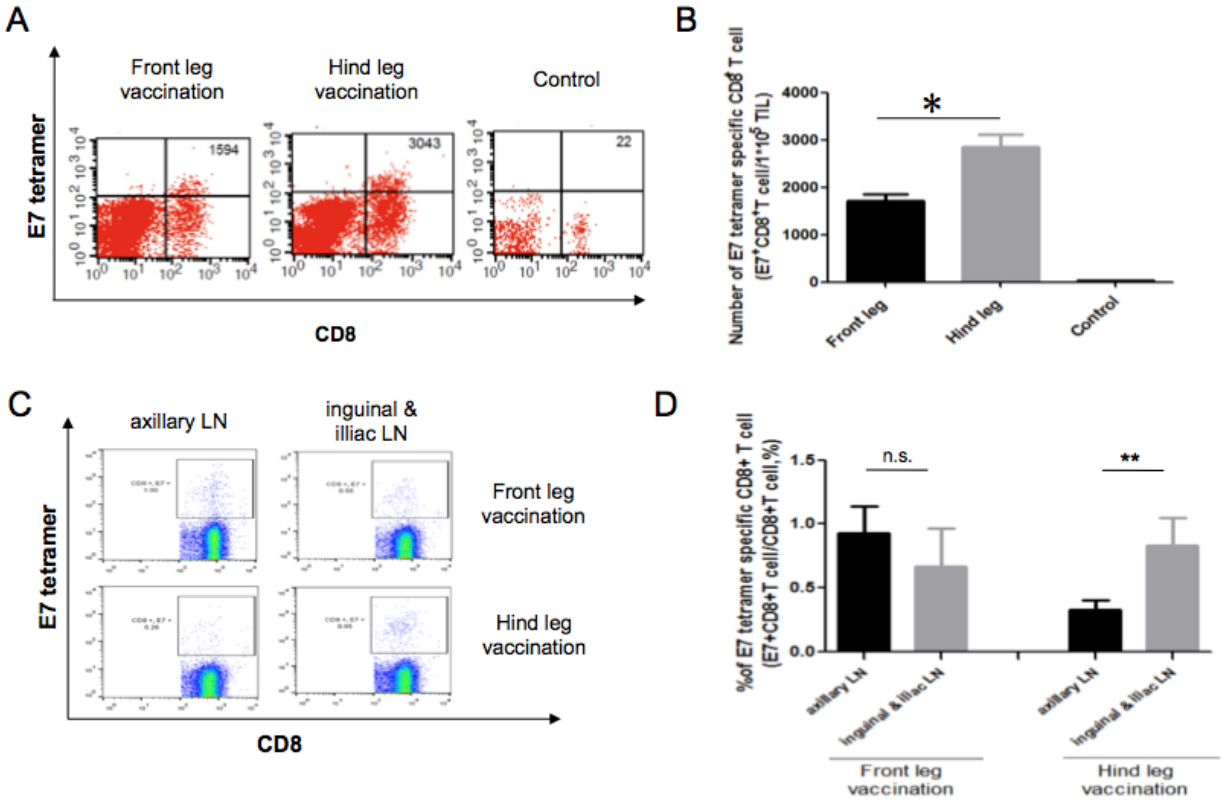


Figure 4. Characterization of E7-Specific CD8+ T cells in tumor and lymph node microenvironment produced by different vaccination sites in TC-1 tumor-bearing mice. 5~8 weeks old female C57BL/6 mice (5 mice/group) were injected with 5×10^4 of TC-1/ luciferase cells at the intravaginal cavity on day 0. The mice were vaccinated with 20 μg/mouse of pNGVL4a-CRT/E7 DNA via intramuscular injection follow by electroporation at different site on day 4 and boosted once 4 days later. 7 days after the last vaccination, lymph nodes and tumors from different groups (both treated and untreated) were harvested, processed, and analyzed for HPV16 E7-specific CD8+ T cells by HPV16 E7 peptide-loaded tetramer staining followed by flow cytometry analysis. **(A-B)** Representative flow cytometry image and Bar graph showing E7-specific CD8+ T cells in TC-1 tumor tissues. **(C-D)** Representative flow cytometry image and Bar graph showing E7-specific CD8+ T cells in inguinal lymph nodes, iliac lymph nodes and axillary lymph nodes. (* = $p < 0.05$, ** = $p < 0.01$, ns = not significant).

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Females, age ≥ 18 years

4.1.2 Diagnosis of HPV16 related stage IB1-IV cervical cancer (**Appendix A**); completed definitive treatment within the past 12 months

4.1.3 No evidence of disease recurrence based on imaging and clinical assessments within 8 weeks of enrollment

4.1.4 Documented to have HPV16 nucleic acid within the cervical tumor specimen as determined by *in situ* hybridization. **NOTE:** HPV16 nucleic acid testing may be done as part of a "pre-screening" consent at any time prior to enrollment on the primary study. To be eligible for HPV16 testing, patients must have stage IB1-IV cervical cancer and have completed definitive treatment such that enrollment within 12 months of diagnosis would be possible if testing is positive.

4.1.5 Fresh-frozen or paraffin-embedded material must be available for *in situ* hybridization testing for HPV16 nucleic acid for central confirmation.

4.1.6 Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 (**Appendix B**)

4.1.7 Adequate organ function as defined by the following parameters:

− white blood cell count	≥ 3,000
− lymphocyte number	≥ 500
− absolute neutrophil count	≥ 1,000
− platelets	≥ 90,000
− hemoglobin	≥ 9
− total bilirubin	<1.5 X upper limit of normal (ULN) (<3 x ULN if Gilbert's disease)
− AST(SGOT)/ALT(SGPT)	<3 X ULN
− creatinine	< 1.5X ULN or estimated creatinine clearance ≥ 60 ml/min per Modified Cockcroft-Gault Formula

4.1.8 Ability to understand and the willingness to sign a written informed consent document

4.1.9 Subject is able to adhere to the study visit schedule and other protocol requirements

4.2 Exclusion Criteria

4.2.1 Patients with a diagnosis of immunosuppression or prolonged, active use of immunosuppressive medications such as systemic steroids

4.2.2 Patients who have had chemotherapy, radiation, biological cancer therapy, or other investigational agents within 28 days prior to the first dose of study drug

4.2.3 Patients who have had surgery within 28 days of dosing of investigational agent, excluding minor procedures (dental work, skin biopsy, etc)

4.2.4 Patients with an uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements

4.2.5 Patients who have an active autoimmune disease (e.g., rheumatoid arthritis, systemic lupus erythematosus (SLE), ulcerative colitis, Crohn's Disease, multiple sclerosis (MS), ankylosing spondylitis)

4.2.6 Patients being chronically treated with immunosuppressive drugs such as cyclosporin, adrenocorticotropic hormone (ACTH), or systemic corticosteroids

4.2.7 Patients with a recognized immunodeficiency disease including cellular immunodeficiencies, hypogammaglobulinemia or dysgammaglobulinemia; patients who have acquired, hereditary, or congenital immunodeficiencies

4.2.8 Women of child-bearing potential (i.e., those who have had fertility-sparing procedures for the management of cervical cancer) will be excluded

4.2.9 Patient with active or chronic infection of HIV, HCV, or HBV (tested at baseline; see Study Calendar)

4.2.10 Patients with non-healed wounds

4.2.11 A history of current or recent concurrent malignancy (≤5 years) except basal cell cancer.

4.2.12 Inability to understand or unwillingness to sign an informed consent document

4.3 Inclusion of Women and Minorities

Only women are eligible for this trial as cervical cancer does not afflict males. Women of all races and ethnic groups are eligible for this trial.

5. REGISTRATION PROCEDURES

5.1 General Guidelines

Pre-Screening: Patients eligible for HPV16 testing must be entered on study centrally at the Sidney Kimmel Comprehensive Cancer Center at the Johns Hopkins University. All sites should call the Lead Study Coordinator/designee at the Coordinating Center. The fax cover sheet, Pre-Screening Registration Form, and Pre-Screening Eligibility Worksheet will be supplied to each participating site.

Primary Study: Eligible patients will be entered on study centrally at the Sidney Kimmel Comprehensive Cancer Center at the Johns Hopkins University. All sites should call the Lead Study Coordinator/designee at the Coordinating Center to verify drug availability. The fax cover sheet, Registration Form, and Eligibility Worksheet will be supplied to each participating site. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5.2 Registration Process

Pre-Screening: To register a patient, the following documents should be completed and faxed or e-mailed to the Coordinating Center:

- Fax Cover Sheet
- Pre-Screening Registration Form
- Signed Patient Consent Form – Pre-Screening
- HIPAA Authorization Form
- Pre-Screening Eligibility Screening Checklist

Once eligibility is verified, to complete the registration process, the Coordinating Center will:

- Assign/confirm a pre-screening patient study number
- Register the patient on the study for pre-screening
- Fax or e-mail the patient study number to the participating site
- Call the research nurse or data manager at the participating site and verbally confirm registration (if needed)

Primary Study: To register a patient, the following documents should be completed and faxed or e-mailed to the Coordinating Center:

- Fax Cover Sheet
- Registration Form
- Signed Patient Consent Form
- HIPAA Authorization Form
- Eligibility Screening Checklist
- Copy of Required Screening Tests and Scans

Once eligibility is verified, to complete the registration process, the Coordinating Center will:

- Assign a patient study number
- Register the patient on the study
- Fax or e-mail the patient study number to the participating site
- Call the research nurse or data manager at the participating site and verbally confirm registration.

6. TREATMENT PLAN

6.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section **8.1**.

There is a possible risk of anaphylaxis and the vaccine should be administered in a setting where emergency treatment is available. Administration should be immediately discontinued and the appropriate therapy instituted per institutional guidelines if there is a serious allergic reaction. The patient must be observed in the clinic for at least 60 minutes after each vaccine. If an anaphylactic reaction is suspected, this must be discussed with the IND Sponsor before the subject is given another dose of vaccine.

6.2 Blinding and Randomization

This is a non-blinded, non-placebo controlled, randomized, prospective study. Eligible patients will be randomized in a 1:1 ratio to receive TA-CIN in either the arm or the thigh. Randomization will occur after registration and prior to the planned first dose of TA-CIN. All participants and providers will know the arm to which subjects are randomized.

A master list of randomization assignments will be generated and will be delivered to the Research Pharmacy at each participating site and to other key personnel, as identified. Upon successful registration of a subject, randomization will occur by the appropriate study staff.

6.3 Dose Levels

The dose of TA-CIN is fixed. The site of administration is as assigned by randomization (as above).

Treatment Groups/Cohorts					
Group	TA-CIN Route of Administration	Volume (mL)	Dose (μg)	Treatment Frequency/Weeks	# of Patients
1	Intramuscular Arm	0.53	100	3 administrations per subject; Weeks 1, 5, and 9	7
2	Intramuscular Thigh				7

6.4 Re-Treatment Criteria

Subjects must maintain the following in order to receive each additional dose of study medication:

• ECOG performance status	0 - 1
• white blood cell count	≥ 3,000
• lymphocyte number	≥ 500
• absolute neutrophil count	≥ 1,000
• platelets	≥ 90,000
• hemoglobin	≥ 9
• total bilirubin	<1.5 X upper limit of normal (ULN) (<3 x ULN if Gilbert's disease)
• AST(SGOT)/ALT(SGPT)	<3 X ULN
• creatinine	< 1.5X ULN or estimated creatinine clearance ≥ 60 ml/min per Modified Cockcroft-Gault Formula

In addition, subjects must not have any acute grade 3 or higher hematologic and/or non-hematological adverse event that is judged possibly, probably or definitely related to the investigational drug agent and/or administration occurring on or after the first day of vaccine administration.

6.5 Dose Modifications and Delays

Dose reduction or dose increase of TA-CIN will not be permitted. The dose in this study is fixed.

In the event that TA-CIN re-treatment criteria are not met, treatment may be delayed for up to 14 days. Future doses should be delayed accordingly such that at least 23 days is maintained between each dose of TA-CIN. The timing for the Month 6, 12, and 24 assessments should be scheduled from the initial dose of TA-CIN.

6.6 Unacceptable Toxicity and Early Stopping Rules

Unacceptable toxicities are defined as treatment-related ≥ grade 4 AEs, or treatment-related grade 3 AEs not improving to ≤ grade 2 under therapy within 2 weeks. Exceptions include: Grade 3 lymphopenia. Because this is a pilot study evaluating safety of immunization, the stopping rule for safety is the presence of vaccine attributable (either probably or definitely) systemic grade 3 or 4 toxicities or grade 4 local/skin toxicities in ≥20% of patients in the study. Patients will be withdrawn from study if they manifest Grade 3 or 4 systemic toxicity attributable to vaccination with TA-CIN or grade 4 local/skin toxicities attributable to vaccination with TA-CIN. In the event of two identical unexpected treatment-related Grade 4 toxicities, accrual will be suspended pending further review.

6.7 General Concomitant Medication and Supportive Care Guidelines

In general, concomitant medications and therapies deemed necessary for the supportive

care and safety of the subject are allowed, provided their use is documented in the medical records. Local vaccine site reaction may be treated with topical applications of aloe vera or vitamin E gel or lotion. Significant local inflammation that is causing the research participant severe pain or is interfering with the activities of daily living may be treated with cold packs and oral analgesics. Local toxicities of pruritus at the vaccine sites and systemic pruritus may be treated with topical or oral diphenhydramine hydrochloride (Benadryl) or topical aloe vera. If oral diphenhydramine hydrochloride is used the recommended dose shall be 25-50 mg every four to six hours as needed for pruritus, not to exceed 300 mg/day. Severe local inflammation or significant clinical autoimmunity will be managed on a case by case basis.

Use the following medications are prohibited during the vaccination administration period and one month (28 days) after last vaccination received:

- Any non-study anticancer or immunotherapy agent (investigational or non-investigational)
- Any other investigational agents
- Live vaccines (examples of live vaccines include, but are not limited to: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid [oral] vaccine). Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed. However, intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.

All concomitant medications will be reviewed and recorded by study staff during the study.

6.8 Contraception, Use in Pregnancy, Use in Nursing

TA-CIN may have adverse effects on an embryo or fetus; therefore, women of childbearing potential with fertility sparing procedures will not be eligible for enrollment.

6.9 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for a total of three vaccinations or until one of the following criteria applies:

- Disease recurrence,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s) (**Section 6.6**),
- Patient or legal representative withdraws consent from the study,
- Patient is lost to follow-up,
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator,
- Non-compliance with protocol or treatment,

- Use of systemic glucocorticoids or other immune-suppressive drugs for more than 7 days required at any time after enrollment in the study, or
- Termination of the study.

6.10 Duration of Follow-Up

Patients will be followed for 24 months after initiation of study treatment or until death, whichever occurs first Patients who are discontinued from the study treatment due to an unacceptable drug-related AE will be monitored for safety until the resolution of the AE to ≤ grade 1 or stabilization or until initiation of a new therapy for their cancer, whichever occurs first.

7. ADVERSE EVENTS

This study will use the descriptions and grading scales found in the revised National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 for adverse event reporting that can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected, recorded, and followed as appropriate.

All adverse events experienced by subjects will be collected and reported from the time of consent, throughout the study, and will only be followed until month 24 unless related to the investigational vaccine. All adverse events related to the vaccine will be followed until resolution.

Subjects who have an ongoing adverse event related to the study procedures and/or medication(s) may continue to be periodically contacted by a member of the study staff until the event is resolved or determined to be irreversible by the investigator.

Laboratory abnormalities: Laboratory abnormalities present at the screening visit will be recorded as pre-treatment signs and symptoms. After study treatment administration, all grade 3 and 4 clinical laboratory results that represent an increase in severity from baseline (pre-study) will be reported as adverse events. A grade 1 or 2 clinical laboratory abnormality should be reported as an adverse event only if it is considered clinically significant by the investigator.

Vaccination site assessment: This study will use the CTCAE version 4.0 injection site reaction grading scale as it relates to vaccine administration associated adverse events. Patients are to have the site of vaccination examined for pain, erythema, warmth, pruritis, induration/fibrosis, and bruising. A follow-up phone call will be made to the patient 7 days after receiving the vaccine to obtain a self-assessment of the vaccination site. The vaccine site(s) will be assessed by the research nurse (or designated clinician) at each subsequent vaccination visit (Day 1 on Weeks 5 and 9) and at the follow-up visit between Weeks 11-13

for local reactions. An optional skin biopsy and/or photographs may be obtained based on the assessment by the principal investigator if the subject has a systemic rash, or an unusual vaccine reaction. In addition to sending this to pathology for diagnosis, we will use additional material from the biopsy for research purposes to understand the effects of the vaccine. We will follow these local reactions until complete resolution.

7.1 Definitions

7.1.1 Adverse Event (AE)

Adverse event is defined as any undesirable sign, symptom or medical condition occurring after starting the study drug (or therapy) even if the event is not considered to be related to the study. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram).

Medical conditions/diseases present before starting the study treatment are only considered adverse events if they worsen after starting the study treatment (any procedures specified in the protocol). Adverse events occurring before starting the study treatment but after signing the informed consent form will be recorded. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy.

7.1.2 Serious Adverse Event (SAE)

A serious adverse event is an undesirable sign, symptom or medical condition which:

- Results in death
- Is life threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below for exceptions) for ≥ 24 hours
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect (note: reports of congenital anomalies/birth defects must also be reported on the Pregnancy Supplemental Form)
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

Events **not** considered to be serious adverse events are hospitalizations for the:

- Admissions as per protocol for a planned medical/surgical procedure
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission for purpose other than remedying ill health state and was planned prior to entry into the study. Appropriate documentation is required in these cases
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative).

The definition of serious adverse event (experience) also includes *important medical event*. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

7.2 Relationship

The Principal Investigator must assess whether AEs have a suspected causal relationship to the study drug. A suspected causal relationship is defined as possibly, probably or definitely related:

Definite – The AE *is clearly related* to the study treatment.
Probable – The AE *is likely related* to the study treatment.
Possible – The AE *may be related* to the study treatment.
Unlikely – The AE *is doubtfully related* to the study treatment.
Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expectedness

Expected adverse events are those which are expected to occur, according to previous clinical experience, and are listed in the Investigator Brochure. The expectedness of an AE will be assessed by the Principal Investigator:

Unexpected adverse event: An adverse event, which varies in nature, intensity or frequency from information on the investigational drug/agent provided in the Investigator's Brochure, package insert or safety reports. Any adverse event that is not included in the informed consent is considered "unexpected".

Expected (known) adverse event: An adverse event, which has been reported in the Investigator's Brochure. An adverse event is considered "expected", only if it is included in the informed consent document as a risk.

7.4 Handling of Expedited Safety Reports

In accordance with local regulations, the IND Sponsor will notify investigators of all SAEs that are unexpected (i.e., not previously described in the IB), and related to the vaccine. This notification will be in the form of an expedited safety report (ESR) that is to be faxed to the investigators and the study coordinators. Upon receiving such notices, the investigator must review and retain the notice with the IB and where required by local regulations, the investigator will submit the ESR to the appropriate IRB. The investigator and IRB will determine if the informed consent requires revision. The investigator should also comply with the IRB procedures for reporting any other safety information.

7.5 Reporting Procedures

7.5.1 General

All adverse events (both expected and unexpected) will be captured on the appropriate study-specific case report forms (CRFs). In addition, all serious adverse events, regardless of causality to study drug and/or administration device, will be reported promptly to the Sponsor (**Dr. Roden,** [redacted]) within 24 hours of recognition of the adverse event (**Appendix D**). If this falls on a weekend or holiday, an email notification is acceptable but must be followed by an SAE reporting form on the next business day. Follow-up information will be submitted to the sponsor as soon as relevant information is available.

7.5.2 Institutional Review Board (IRB)

All serious adverse events will be reported to the Institutional Review Board (IRB) per institutional standards. If a serious adverse event requires modification of the study protocol and informed consent, these modifications will be provided to the IRB with the report of the adverse event. Follow-up information will be submitted to the IRB as soon as relevant information is available per institutional standards.

7.5.3 Food and Drug Administration (FDA)

7.5.3.1 Expedited IND Safety Reports:

7 Calendar-Day Telephone or Fax Report:
The Sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be at least possibly related to the investigational agent. Such reports are to be telephoned or faxed [redacted] to the FDA within 7 calendar days of first learning of the event. Follow-up information will be submitted to the FDA as soon as relevant information is available.

15 Calendar-Day Written Report:
The Sponsor is required to notify the FDA of any serious adverse event that is unexpected and possibly related to the investigational agent in a written IND Safety Report.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA within 15 calendar days of first learning of the event. Follow-up information will be submitted to the FDA as soon as relevant information is available.

7.5.3.2 IND Annual Reports

In accordance with the regulation 21 CFR § 312.33, the Sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the adverse events and progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.33 for a list of the elements required for the annual report. All IND annual reports will be submitted to the FDA by the Sponsor-Investigator.

8. PHARMACEUTICAL INFORMATION

Please refer to the Laboratory Manual for further details regarding storage, preparation, and administration of the product.

8.1 TA-CIN

Product Description: The final formulated product TA-CIN (HPV16 L2E7E6 fusion polypeptide) contains 0.19 mg/mL TA-CIN in 5mM phosphate, 5mM glycine, 0.9mM cysteine at pH8.1 ± 0.3. The product is supplied by the Waisman Biomanufacturing (Madison, WI). The vaccine will be supplied in 0.65mL aliquots.

Storage Requirements: Vaccine supply is to be stored at ≤ -20°C.

Solution Preparation: Thaw individual vials at ambient temperature. TA-CIN should be removed from –20°C storage and allowed to defrost at room temperature for up to 30 minutes. **Do not refreeze once defrosted.** Gently invert the vial 5-6 times before dose is drawn up. Using a 25G (orange) or 23G (blue) needle and 1mL syringe, remove 0.53 mL of the clear solution. Administer a total volume of 0.53 mL by intramuscular vaccination within 2 hours of drawing up into the syringe.

Stability: Administer a total volume of 0.53 mL by intramuscular vaccination within 2 hours of drawing up into the syringe.

Route of Administration: The vaccine will be administered via one of two injection sites: 1) Intramuscular needle injection in the non-dominant medial deltoid (arm) region

using a 25 gauge needle, or 2) Intramuscular needle injection in the Vastus Lateralis (thigh) region using a 23 or 25 gauge needle. In the event that the specified arm or thigh is contraindicated, the other arm or thigh may be used respectively. A patient will receive all 3 injections in the arm if randomized to this group, or all 3 injections in the thigh if randomized to the alternate group.

Known Adverse Effects: TA-CIN has been administered in over 90 adults (healthy adults and high grade VIN or AGIN 3 patients) across four clinical trials. To date, no patients with a history of advanced cervical cancer have received the TA-CIN vaccine. Based on the previous clinical safety data for TA-CIN, the following adverse events are expected when administering the TA-CIN vaccine:

System/Organ	Adverse Event	
Blood/Lymphatic	Eosinophilia	
Constitutional	Chills Fatigue Fever	Flu-like symptoms Flushing Somnolence
Dermatologic	Rash	Urticaria
Gastrointestinal	Abdominal Pain Anorexia	Nausea Vomiting
HEENT	Dry Mouth	Rhinitis
Neurology	Dizziness Dreaming Abnormal	Headache Syncope
Vaccine Site Reactions	Bruising Erythema Pain	Pruritis Swelling Tenderness
Other	Arm Weakness Hypotension Menstrual Disorder	Myalgia Pain in both arms

9. CORRLEATIVE STUDIES

Please refer to the Laboratory Manual for further details regarding collection, processing and handling of specimens for the correlative studies.

9.1 Blood Samples for Immunologic Assays and Viral DNA

Blood will be drawn at various time schedules as noted in the protocol. The volume of blood and container utilized will vary depending on the test to be performed, as detailed below:

9.1.1 **HPV16 L2, E6, E7 and HPV Neutralizing:** Blood (1, 10cc SST [serum separator tube]) will be utilized for measurement of antibodies to HPV16 L2, E6, and E7 via

ELISA per routine laboratory methods and also the HPV in vitro neutralization assay.

9.1.2 **HPV16 E6- and E7-Specific T cells:** Blood (1, 60cc heparinized syringe) will be collected to evaluate HPV16 antigen-specific T cell responses at the indicated time points during the vaccination series.

9.1.3 **Evaluation of the Proliferative Responses of Peripheral Blood Lymphocytes to Stimulation by HPV16 E6 and E7:** Evaluation of the proliferative responses of peripheral blood lymphocytes to stimulation by HPV16 E6 and E7 using heparinized blood sample (see 9.1.2).

9.1.4 **Evaluation of HPV16 DNA in Plasma**. Plasma and cells will be separated from blood (1, 10cc EDTA) by standard protocol and stored at –70 ºC until processing. HPV DNA load in plasma samples will be quantified by use of real-time quantitative PCR and T cell receptor sequencing performed.

9.2 Samples for HPV16

Tissue will be sent as part of a "pre-screening" consent process for eligibility purposes, as detailed below:

9.2.1 **HPV16 *in situ* Hybridization and p16 Immunohistochemistry Testing:** Fresh-frozen or paraffin-embedded material from the cervical cancer tumor specimen must be available for testing to confirm eligibility if this was not done per routine care. Samples will be sent to Johns Hopkins for analysis in a CLIA-certified environment and results will be returned to the study team/provider (**Appendix C**). Sites will be provided with instructions on how to send samples to Johns Hopkins for testing at time of activation.

In the event that standard of care HPV16 testing was done and is positive, and is felt to be adequate by the Protocol Chair, repeat testing will not be needed for eligibility purposes prior to screening and enrollment on the primary treatment study. In these cases archival tissue specimens will still be obtained for research analyses.

9.3 Other Assessments

Disease Status Evaluation: After completion of standard therapy, disease status will be determined by current standard of care. Disease assessment after vaccine administration is to be performed as per current clinical care standards. Imaging studies, specifically a PET/CT, CT, or MRI scan (whichever is clinically indicated and/or preferred by the treating physician), will be performed per standard of care and/or based on the discretion of the treating physician.

10. STUDY CALENDAR

Test/Procedure	Pre-Study[17]	Week 1 Day 1	Week 2 Day 1	Week 5 Day 1	Week 6 Day 1	Week 9 Day 1	Week 10 Day 1	Week 11-13	Month 6	Month 12	Month 24
Visit Window (Days)	-60		+/- 2	+/- 5	+/- 5	+/- 5	+/- 5		+/- 28	+/- 28	+/- 28
TA-CIN Administration[1]		X		X		X					
Tumor HPV Testing[2]	X										
Informed Consent	X										
Demographics	X										
Medical History	X										
Class I and II HLA Typing[3]	X										
HIV/Hepatitis Panel[4]	X										
Physical/Pelvic Examination[5]	X	X		X		X		X	X	X	X
Vital Signs[6]	X	X		X		X		X	X	X	X
Performance Status[7]	X	X		X		X		X	X	X	X
Concomitant Med Review	X	X		X		X		X	X	X	X
Vaccine Site Assessment[8]				X		X		X			
Phone Call			X		X		X				
CBC w/Diff, Plts[9]	X	X		X		X		X	X	X	X
Serum Chemistry[10]	X	X		X		X		X	X	X	X
PET/CT, MRI or CT Scan[11]	X										
Evaluation of HPV16 E6, E7-Specific $CD8^+$ T Cells[12]	X[13]			X[13]		X[13]		X[13]	X[13]	X[13]	X[13]
Evaluation of HPV16 Antibody and DNA[15] and HPV16 Viral Load	X[14]			X[14]		X[14]		X[14]	X[14]	X[14]	X[14]
Adverse Event Evaluation[15]		→	→	→	→	→	→	→			
Archived Tumor Tissue[16]	→	→	→	→	→	→	→	→	→	→	→

Research samples will be collected at the discretion of the PI based on availability of supplies and safety of patient and staff.

In order to minimize the need for research-only in-person visits, telemedicine visits may be substituted for in-person clinical trial visits or portions of clinical trial visits where determined to be appropriate and where determined by the investigator not to increase the participants risks. Prior to initiating telemedicine for study visits the study team will explain to the participant, what a telemedicine visit entails and confirm that the study participant is in agreement and able to proceed with this method. Telemedicine acknowledgement will be obtained in accordance with the Guidance for Use of Telemedicine in Research. In the event telemedicine is not deemed feasible, the study visit will proceed as an in-person visit. Telemedicine visits will be conducted using HIPAA compliant method approved by the Health System and within licensing restrictions.

1: Administration site (arm or thigh), as assigned.
2: Formalin fixed paraffin embedded tumor sections will be tested for HPV16 DNA/RNA *in situ* hybridization and p16 immunohistochemistry.
NOTE: This may be done as part of a pre-screening consent process and/or at any time prior to enrollment to the main study. Otherwise, there is no window for HPV16 testing for eligibility. Sites without the capability to conduct HPV16 testing will submit slides from archival tissue to Johns Hopkins
3: Peripheral blood will be drawn prior to Week1 Day 1 dosing to undergo HLA typing at study site.
4: HIV, Hepatitis C Antibody, and HBsAg. Consent will be obtained for HIV testing.
5: Complete physical exam, including pelvic exam, will be completed at baseline (pre-study); focused physical examinations will be conducted thereafter. The Week 1 Day 1 physical examination does not need to be completed if the screening physical examination was performed ≤ 7 days prior.
6: Including (1) heart rate; (2) respiratory rate; (3) body temperature (°C), (4) height (centimeters), and (5) weight (kilograms). Height is only to be obtained once during pretreatment evaluation.
7: Eastern Cooperative Oncology Group performance status, 0 to 5.
8: Vaccine site reactions include, but are not limited to: erythema, induration, pruritis, tenderness, warmth, blisters, and vaccine site flares. Vaccine site reactions should be followed until resolution.
9: Sodium, potassium, bicarbonate, chloride, blood urea nitrogen, serum creatinine, glucose, magnesium, calcium, total bilirubin, direct bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, albumin, total protein. Labs may be collected up to 3 days prior to dosing.
10: CBC with differential including absolute eosinophil count, absolute neutrophils, absolute lymphocytes, and platelets. Labs may be collected up to 3 days prior to dosing.
11: PET/CT or CT of the chest/abdomen/pelvis at baseline (within 8 weeks) and as clinically indicated. If a subject cannot have a CT scan (e.g., allergy to contrast dye), a non-contrast enhanced PET/CT scan or MRI should be performed. In the event the patient cannot undergo CT, PET/CT, or MRI, a Chest X-Ray should be performed.
12: Patients will undergo phlebotomy for the detection of E6 and E7-specific $CD8^+$ T cells.
13: 60 cc of whole blood

14: 10cc of blood for serum (antibody assays) and 10cc of blood for plasma (for measurement of HPV16 DNA).
15: Adverse events will be evaluated up to 30 days after the last vaccination. Any adverse events related to the vaccine will be followed until resolution or until investigator deems the adverse event as chronic and/or stable. An optional skin biopsy may be performed for any systemic rash or vaccine reaction at the site of administration.
16: Attempts to obtain additional archival tumor samples in excess to diagnosis that were collected as part of routine clinical care.
17: Pre-Study CBC with differential, platelets and serum chemistry do not need to be repeated in Week 1 if done ≤ 7 days prior

11. DATA AND SAFETY MONITORING

Adverse event lists, guidelines, and instructions for AE reporting can be found in **Section 7.0** (Adverse Events: List and Reporting Requirements).

11.1 Data Management

All information will be collected on study-specific case report forms (CRFs) by study staff. These data will be reviewed for completeness and accuracy by the Principal Investigator as well as the Sidney Kimmel Comprehensive Cancer Center Clinical Research Office.

Protocol Chair

The Protocol Chair is responsible for performing the following tasks:

- Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.
- Assuring that all participating institutions are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.
- Reviewing and ensuring reporting of Serious Adverse Events (SAE)
- Reviewing data from all sites.

Coordinating Center

The Coordinating Center is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.
- Managing central patient registration.
- Collecting and compiling data from each site.
- Establishing procedures for documentation, reporting, and submitting of AE's and SAE's to the Protocol Chair, and all applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites for audit, or by auditing at participating sites.

Participating Sites

Participating sites are responsible for performing the following tasks:

- Following the protocol as written, and the guidelines of Good Clinical Practice (GCP).
- Submitting data to the Coordinating Center (at the request of the Coordinating Center and the frequency dependent on the rate of subject accrual and the progress of the study).
- Registering all patients with the Coordinating Center by submitting patient registration form, and signed informed consent promptly.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.

- Maintaining regulatory binders on site and providing copies of all required documents to the Coordinating Center.
- Collecting and submitting data according to the schedule specified by the protocol.

11.2 Safety Meetings

Scheduled meetings will take place weekly and will include the protocol principal investigator, study coordinator(s), data manager(s), sub-investigators (as appropriate), collaborators (as appropriate), and biostatisticians (as appropriate) involved with the conduct of the protocol. During these meetings, matters related to the following will be discussed: safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for objectives.

11.3 Monitoring

Interim analysis of toxicity, outcome and ongoing scientific investigations will be performed every 6 months by the Sidney Kimmel Comprehensive Cancer Center Data Safety Monitoring Board (SKCCC DSMB). The SKCCC DSMB will review aspects of this trial that are outlined in the responsibilities section of the Data and Safety Monitoring Board (DSMB) Guidance. If the committee decides that amendments should be made to this trial, recommendations will be made in writing to the Study Principal Investigator. The study team will submit modifications to the IRB within 60 days of receipt from the DSMB. The Associate Director of Clinical Research, will arbitrate any disagreements between the DSMB and the study Principal Investigator. These changes may include early termination of accrual if deemed appropriate.

Johns Hopkins SKCCC: The protocol will be monitored externally by the SKCCC CRO in accordance with SKCCC guidelines. Trial monitoring and reporting will be done through the Safety Monitoring Committee (SMC) at SKCCC.

Participating site(s): The protocol will be monitored by the internal CRO at each site. A report of the reviews will be submitted to the Johns Hopkins principal investigator and SKCCC CRO.

Authorized representatives of the Coordinating Center may visit the satellite sites to perform audits or inspections, including source data verification. The purpose of these audits or inspections is to systematically and independently examine all trial-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP), and any applicable regulatory requirements.

The Medical Expert Committee (MEC) for this clinical study contains three medical oncologists (see below) from other disciplines who are not affiliated with this clinical trial protocol. The MEC will review safety data on at least a semi-annual basis. The MEC

will provide a written summary of each assessment to the IND Sponsor after each meeting. In turn, the study team will forward these summaries to the JHU IRB, and JHU SKCCC SMC. The operating plan of the MEC will be as follows:

- Meetings will be held at least semi-annually, and potentially more frequently if needed.
- Meetings will be conducted in-person or via video/teleconference, with a participant sign-in sheet collected at each meeting.
- Approximately one week prior to each MEC meeting, the study team will submit the following items to MEC personnel for review and discussion at the meeting (The PI may join the MEC meeting in order to answer any questions the MEC might have):
  - A summary of the clinical trial's progress to date;
  - The latest IRB-approved consent document; and,
  - A summary of all adverse events, serious adverse events, deaths, and withdrawals to date.

Note that the MEC reserves the right to halt trial accrual or all study activity if, after review, serious safety concerns warrant this action. If the MEC halts study accrual or all study activity, then the study team must notify the JHU SKCCC SMC, JHU IRB, and the FDA immediately.

Dr. Roden will be holding the IND for this study. He will comply with all regulated reporting requirements to the FDA.

12. STATISTICAL CONSIDERATIONS

12.1 Study Design and Endpoints

This pilot study is a single dose level assessment of the immunologic response as well as the safety and tolerability of administering TA-CIN vaccine via different sites in patients who have a history of HPV16-related cervical cancer. The primary goal is to determine the most favorable injection site for TA-CIN that exhibits the greater HPV-specific immunogenicity. The secondary goal is to evaluate the safety and tolerability profile of TA-CIN in HPV16 related cervical cancer patients.

The primary endpoint is the immune response rate at the time of the fourth follow-up measurement. The measure of immune response to the regimen will be the antigen-specific T cell response, the simulation index of T cell proliferation, and the titer of the antibody response in peripheral blood. Each patient will have one measurement at baseline and 4 measurements at 4 time points after treatment. For each individual patient, a significant immunologic response is defined as the maximum increase in antigen-specific T cell number or proliferation index after administration of TA-CIN by greater than 1.8 times the standard deviation (SD) of the baseline values, and the increase is at least 2-fold. An increase by 1.8 SD establishes a statistically significant reduction at the two-sided .10 level.

This study will evaluate two different sites, thigh and arm, for TA-CIN vaccine administration.

12.2 Sample Size

A total of 15 patients with a history of advanced HPV16-related cervical cancer will be enrolled and randomized at 1:1 ratio to receive TA-CIN administrations via thigh (Group A) or arm (Group B). Each group will treat at most 7 patients. A previous study by Smyth *et al*. in non-cancer patients who did not receive chemo/radiotherapy showed that 9 out of 25 patients had a detectable E6/E7-specific T cell response one month after TA-CIN vaccination. It is expected that the prior chemo/radiotherapy received by the cervical cancer patients enrolled on this study will prime the response to the TA-CIN such that the T cell response rate will be higher. If the true response rate is 65% for Group A and 45% for Group B, with 7 patients each group, there is about 70% chance of picking the correct injection site based on the observed number of responders in the two groups. Even if the true response rate is 60% for Group A and 45% for Group B, with 7 patients each group, there is still about 60% chance to correctly pick the winner.

12.3 Accrual Rate

The Kelly Gynecologic Oncology service at JHU sees ~50 cervical cancer cases per annum and UAB 120 per annum. Assuming half are HPV16+ and 15% are eligible to join the study then enrollment using both sites should be completed in 1 year.

12.4 Early Stopping Rules

This study will evaluate two different sites of TA-CIN vaccine to determine the safety

and effectiveness of TA-CIN in patients with a history of advanced cervical cancer. For each injection site, the SAE rate of 20% or more is deemed unsafe. A total of 15 patients will be enrolled, with 7-8 patients in each injection site cohort.

The study will be stopped early if there are more than two patients with SAEs observed during the study. If the SAE rate is 20% or more, there is at least 55% chance to stop the study early with 15 patients.

12.5 Statistical Analysis

Adverse events will be tabulated by type and grade for each dose cohort.

The measure of immune response to the regimen will be the number of antigen-specific T cells detected by ELISPOT and/or ICC, or the simulation index of T cell proliferation, and the titer of the serum antibody as determined by ELISA. Each patient will have one measurement at baseline and 5 measurements at 5 time points after treatment. Descriptive statistics (mean and SD) and box plots will be used to summarize the immunogenicity by time point and dose cohort. Formal hypothesis testing will not be conducted to determine the ideal vaccination site. Instead, a "winner" will be picked based on the observed number of responses (*42*). Two-sample t-test will be used to compare the immunogenicity between two immunization sites. Data transformation will be applied if the distribution is not normal. In addition, the time course of outcomes will be investigated graphically by individual subject plots.

Cox regression analysis will be conducted to explore the association between time to disease recurrence and vaccine-induced immune response status. Patient baseline characteristics including disease stage and history of treatment will be adjusted in the analysis.

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APPENDICES

APPENDIX A: FIGO 2009 Cancer Staging

Carcinoma of the Cervix

IA1 Confined to the cervix, diagnosed only by microscopy with invasion of < 3 mm in depth and lateral spread < 7 mm

IA2 Confined to the cervix, diagnosed with microscopy with invasion of > 3 mm and < 5 mm with lateral spread < 7mm

IB1 Clinically visible lesion or greater than A2, < 4 cm in greatest dimension

IB2 Clinically visible lesion, > 4 cm in greatest dimension

IIA1 Involvement of the upper two-thirds of the vagina, without parametrial invasion, < 4 cm in greatest dimension

IIA2 > 4 cm in greatest dimension

IIB With parametrial involvement

IIIA Tumor involves lower third of the vagina with no extension to the pelvic side wall

IIIB Tumor involves extension to the pelvic side wall and or hydronephrosis or nonfunctioning kidney

IVA The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum. A bullous edema, as such, does not permit a case to be allotted to stage IV.

IVB Spread to adjacent organs

APPENDIX B: Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active 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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX C: Pathology Review

Study Number: _____

Patient ID: _____

Specimen ID Number: _____

Date Specimen Obtained: _____

Study Site: _____

Person Sending to Pathologist: _____ Initials: _____

Quality of Tissue (H&E): ☐ Satisfactory ☐ Not satisfactory

Microscopic Diagnosis:

☐ No Evidence of *in situ* or Invasive Carcinoma or SIL
☐ Adenocarcinoma *in situ*
☐ Invasive Adenocarcinoma
☐ LSIL
☐ HSIL
☐ Invasive Squamous Cell Carcinoma
☐ Other: _____

Diagnostic Testing:

☐ **p16 Immunohistochemistry:**
Performed: ☐ Yes ☐ No
Quality of Staining: ☐ Satisfactory ☐ Not Satisfactory
Result: ☐ Positive (Diffuse Expression in 90-100% of Lesional Cells) ☐ Negative (Non-Diffuse Pattern)

☐ **HPV16 *in situ* Hybridization:**
Performed: ☐ Yes ☐ No
Quality of Staining: ☐ Satisfactory ☐ Not Satisfactory
Result: ☐ Positive ☐ Negative For HPV16

Study Pathologist: _____ _____ _____
Print Name Signature Date

Fax Completed Form ASAP to Dr. Stephanie Gaillard (Study PI) [redacted]

Pathologist's Recommendations:

☐ No further work up required: proceed with study.
☐ Possible work up required: review and discuss patient in multidisciplinary conference.
☐ Definite work up required: allocate specimen for official review by the department of pathology; review and discuss patient in multidisciplinary conference.

Site Investigator: _____ _____ _____
Print Name Signature Date

Subject Notified By: _____ Date: _____

Comments: _____

APPENDIX D: Serious Adverse Event Reporting Form

****Must Notify Dr. Roden Within 24 Hours of Study Team Knowledge of Serious Adverse Events****

<table>
<tr><td>Protocol Title:</td><td colspan="3">A Phase I Clinical Trial Assessing the Safety and Feasibility of Administration of the TA-CIN Vaccine in HPV16 Associated Cervical Cancer Patients</td></tr>
<tr><td rowspan="2">Report Type:

☐ Initial
☐ Follow-up #___
☐ Death
☐ Addendum to:
_____</td><td rowspan="2">Serious Criteria (Check All That Apply):
☐Death
☐Life-Threatening
☐Hospitalization or Prolongation of Existing Hospitalization
☐Persistent or Significant Disability
☐Congenital Anomaly
☐Other Important Medical Event</td><td>Adverse Event Onset Date:</td><td>Date of Report:</td></tr>
<tr><td>Adverse Event End Date:</td><td>Date Event Discovered:</td></tr>
<tr><td colspan="4">Section A: Subject Information</td></tr>
<tr><td>Subject ID:
Subject Initials:</td><td colspan="2">Subject Age at Event Onset:</td><td>Subject Gender:
☐Male
☐Female</td></tr>
<tr><td colspan="4">Section B: Event Information</td></tr>
<tr><td rowspan="2">Event Diagnosis Or Symptom(s):</td><td colspan="2">Date of 1st Dose (TA-CIN):</td><td rowspan="3">Action Taken With The Study Drug (TA-CIN):

☐None
☐Interrupted
☐Discontinued
☐Delayed</td></tr>
<tr><td colspan="2">Date of 2nd dose (TA-CIN):</td></tr>
<tr><td>CTCAE Severity:

☐ Grade 1 (Mild)
☐ Grade 2 (Moderate)
☐ Grade 3 (Severe)
☐ Grade 4 (Life-Threatening)
☐ Grade 5 (Death)</td><td colspan="2">Date of Last Dose (TA-CIN) Prior to Event:</td></tr>
<tr><td>Outcome:

☐ Ongoing
☐ Recovering/Resolving
☐ Recovered/Resolved
☐ Recovered/Resolved with Sequelae (specify): _____
☐ Fatal
☐ Unknown</td><td colspan="2">Number of Total Doses (TA-CIN):</td></tr>
<tr><td colspan="2">Hospital Admission Date:</td><td colspan="2">Hospital Discharge Date:</td></tr>
</table>

Relationship to:	TA-CIN Vaccine	Underlying Disease	Other Causality _____ *(Please Specify)*
Unrelated	☐	☐	☐
Probably Unrelated	☐	☐	☐
Possible Related	☐	☐	☐
Probably Related	☐	☐	☐
Definitely Related	☐	☐	☐

Section C: Brief Description of the Event (*Attach Additional Pages as Necessary*)

Section D: Relevant Medical History (*Attach Additional Pages as Necessary*)

Section E: Concomitant Drug (Taken Prior to SAE Onset) (*Attach Additional Pages as Necessary*)

Name of the Drug *(Trade or Generic)*	Start Date	Stop Date	Route	Dose	Frequency

Section F: Relevant Labs (*Attach Additional Pages as Necessary*)				
Lab Test Name	**Date**	**Value**	**Unit**	**Reference Range**

Section G: Comments (*Attach Additional Pages as Necessary*)

Section G: Additional Documents (*Attach Additional Pages as Necessary*)
☐ Please specify:

Reporter's Name:	**Reporter's Signature:**
Phone Number:	**Signature Date:**
Investigator's Name:	**Investigator's Signature:____**
Phone Number:	**Signature Date:**