A Phase I/II Clinical Trial of PepCan in Head and Neck Cancer Patients in Remission to Reduce Recurrence Regardless of HPV Status

Version 19

January 5, 2024

IRB: 217672

IND 15173

NCT Number: NCT03821272

UAMS Principal Investigator:	Omar Atiq, MD Distinguished Professor of Medicine and Otolaryngology-Head and Neck Surgery Winthrop P. Rockefeller Cancer Institute, College of Medicine, University of Arkansas for Medical Sciences 4301 W. Markham St., Slot 508 Little Rock, AR 72205 (501) 686-8530 or after hours at (501) 686-6080 *OTAtiq@uams.edu*
Medical Monitor:	Rangaswamy Govindarajan, MD Professor, Dept. Internal Medicine, Hematology-Oncology College of Medicine University of Arkansas for Medical Sciences *GovindarajanRang@uams.edu*
Study Statistician:	Milan Bimali Associate Professor, Dept. Health Biostatistics College of Public Health University of Arkansas for Medical Sciences *MBimali@uams.edu*
Sponsor:	University of Arkansas for Medical Sciences (UAMS)
Funding Source:	UAMS/National Institutes of Health (National Cancer Institute)

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ABBREVIATIONS

AE	Adverse Event
Alk Phos	Alkaline Phosphate
ALT	Alanine Aminotransferase Test
AR-AERS	AR-Adverse Event Reporting System
AST	Aspartate Aminotransferase Test
BUN	Blood Urea Nitrogen
cGMP	Current Good Manufacturing Practice
CBC with diff	Complete Blood Count with Differential
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CIN	Cervical Intraepithelial Neoplasia (Grade 1, 2, or 3)
CCTRA	Cancer Clinical Trials Regulatory Affairs
CLARA	Clinical Research Administrator
Chem 7	Basic Metabolic Panel: Sodium, Potassium, Chloride, Carbon Dioxide, Anion Gap, BUN, Creatinine, eGFR, Calcium
CO_2	Carbon Dioxide
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DNA	Deoxyribonucleic Acid
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EDTA	Ethylenediaminetetraacetic Acid
eGFR	estimated Glomerular Filtration Rate
ELISPOT	Enzyme-Linked Immunospot
ENT	Ear, Nose, Throat
FACS	Fluorescence-Activated Cell Sorting
FDA	United States Food and Drug Administration
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HNSCC	Head and Neck Squamous Cell Carcinomas
HPV	Human Papillomavirus
HPV-16	Human Papillomavirus Type 16
HSIL	High Grade Intraepithelial Neoplasia
ICF	Informed Consent Form
IFN-γ	Interferon-γ
IND	Investigational New Drug Application
IRB	Institutional Review Board
LD	Lactate Dehydrogenase
LEEP	Loop Electrical Excision Procedure
LFTs	Liver Function Tests: Total Bili, Alk Phos, AST, ALT, LD
MDSC	Myeloid-Derived Suppressor Cells
NED	No Evidence of Disease

NIH	National Institutes of Health
NCI	National Cancer Institute
OC	OpenClinica
ORRA	Office of Research Regulatory Affairs
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
RPRS	Research Participation Registration System
SAE	Serious Adverse Event
SIL	Squamous Intraepithelial Lesion
Th1	T-helper Type 1
Th2	T-helper Type 2
Total Bili	Total Bilirubin
UAMS	University of Arkansas for Medical Sciences
VLP	Virus-Like Particles

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with all applicable United States (US) Code of Federal Regulations (CFR) The Principal Investigators (PIs) will assure that no deviation from, or changes to the protocol will take place without prior agreement from the funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial subjects. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form will be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; the IRB and/or Sponsor will determine whether subjects need to be re-consented.

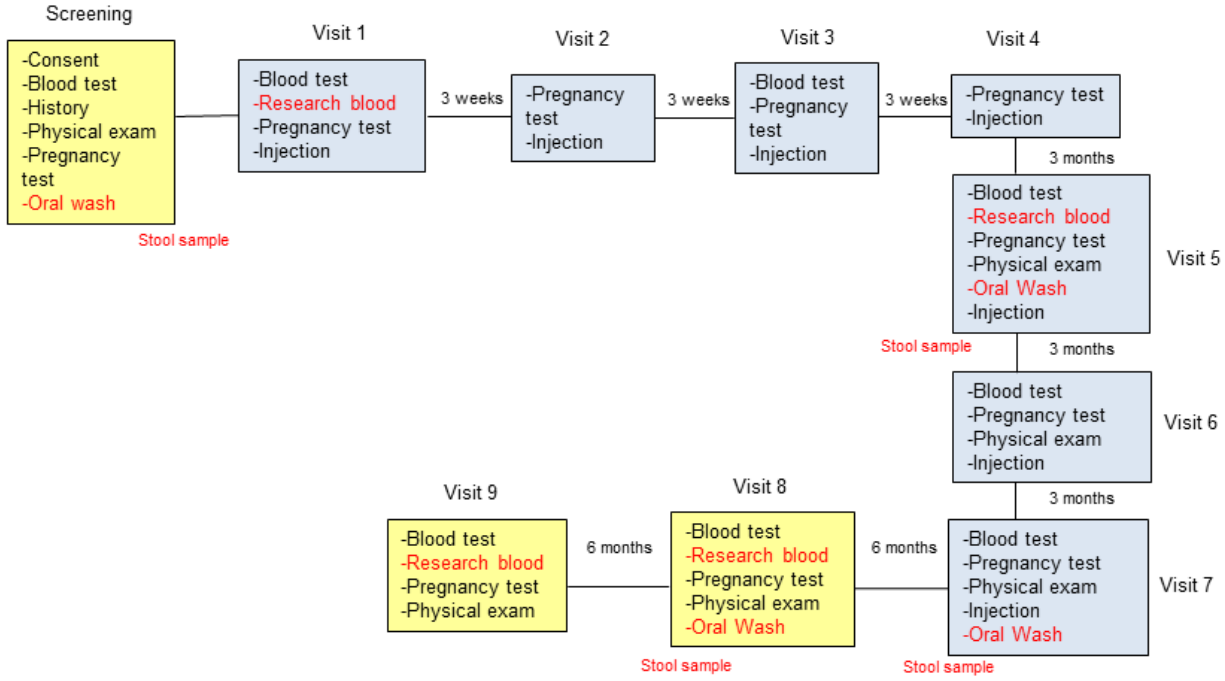
1. PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	A Phase I/II Clinical Trial of PepCan in Head and Neck Cancer Patients in Remission to Reduce Recurrence Regardless of HPV Status
IRB Number:	217672
Study Description:	This is a Phase I/II study to evaluate the efficacy and safety of an HPV therapeutic vaccine called PepCan (HPV-16 E6 peptides) in adults over a two (2) year period. Each subject will be receiving a total of 7 injections of PepCan (50 µg per peptide dose) or placebo (saline) at a 3:1 ratio in a randomized double-blinded design. Subjects will receive one injection every 3 weeks until they receive 4 injections. Then, subjects will receive one injection every 3 months until they receive a total of 7 injections. Subjects will have 2 more visits approximately 6 months apart after the last injection. Immunological assessment by enzyme-linked immunospot (ELISPOT) assay and by fluorescent activated cell sorter analysis (FACS) will be made at 4 time points (Visits 1, 5, 8, and 9). Stool samples will be collected at the Screening Visit, Visit 5, Visit 7 and Visit 8 for microbiome diversity analysis.
Objectives:	Primary Objective: To evaluate the safety of a 7-injection regimen of PepCan. Secondary Objective: To evaluate the efficacy of a 7-injection regimen of PepCan by observing cancer recurrence. Tertiary Objective: To evaluate the immunological response to PepCan. Quaternary Objective: To analyze microbiome profile of DNA in subjects receiving PepCan.
Endpoints:	Primary Outcome Measure: Safety of 7 dose regimen as defined by adverse events (AEs) after 2 years Secondary Outcome Measure: Efficacy of 7 dose regimen by clinically observing cancer recurrence Tertiary Outcome: Immunological responses assessed using ELISPOT assay and FACS analysis Exploratory Outcome: Profile of gut and oral microbiome before and after vaccination
Study Population:	One hundred (100) subjects, 18 years of age or older, with a head and neck cancer diagnosis who have achieved complete remission, regardless of HPV status
Phase:	Phase I/II
Description of Study Intervention:	The vaccine arm will receive seven injections of PepCan; the placebo arm will receive seven injections of an equal volume of placebo Route of Administration: Intradermal injection (limb) Peptide Dose Level: 50 µg/peptide/injection Adjuvant dose level: 0.3 mL Candin®/injection Placebo: Intravenous 0.9% NaCl solution (Saline) Dosing Regimen: 7 injections total, one injection every three weeks for the first 4 injections. Then one injection every 3 months until a total of 7 injections has been administered.

Participation Duration:	From the first day of vaccine administration subjects will be followed for two (2) years.
Statistical Methodology:	Measures of safety will be evaluated using descriptive statistics. Cancer recurrence rate will be compared between the PepCan and placebo arm.

1.2 SCHEMA



The items shown in red are samples to be shipped to UAMS.

2. INTRODUCTION

2.1 BACKGROUND

Head and neck cancers account for approximately 4% of all cancers in the United States [1]. These cancers are more than twice as common among men as they are among women [2]. Head and neck cancers are diagnosed more often among people over age 50 than they are among younger people. Researchers estimated that more than 65,000 men and women in this country would be diagnosed with head and neck cancers in 2017 [2]. A large number of these cases are associated with a patient's use of alcohol and tobacco. However, the incidence of cases related to a human papilloma virus (HPV) infection, is growing [3, 4]. Recent studies have shown 70% of oropharynx, 25% of head and neck squamous cell carcinomas (HNSCC), 24% of laryngeal, and 23% of oral cavity cancers are linked to HPV [4].

Human papilloma viruses are a group of more than 200 related viruses. More than 40 HPV types can be easily spread through direct sexual contact, from the skin and mucous membranes of infected people to the skin and mucous membranes of their partners. About 14 million new genital HPV infections occur each year [4]. The Centers for Disease Control and Prevention (CDC) estimates that more than 90% and 80% of sexually active men and women, respectively, will be infected with at least one type of HPV at some point in their lives [5-7]. Around one-half of these infections are with a high-risk HPV type [7].

High-risk HPVs can cause cancer. About a dozen high-risk HPV types have been identified. Two of these, HPV types 16 and 18, are responsible for most HPV-caused cancers [4, 6]. HPV type 16 is associated with 90% of HNSCC associated with HPV.

High-risk genotypes of HPV, specifically type 16, are found in a discrete subset of HNSCC. Cases of HPV-related HNSCC may be prevented with vaccines. Inducing appropriate HPV virus specific immune responses may aid in prevention and treatment efforts. Infection by HPV may be averted by neutralizing antibodies specific for the viral capsid proteins. In clinical trials, vaccines comprised of HPV virus-like particles (VLPs) have shown great promise as prophylactic HPV vaccines. However, given that capsid proteins are not expressed at detectable levels by infected basal keratinocytes, vaccines with therapeutic potential must target other non-structural viral antigens [8].

HPV transformation of squamous epithelium to a malignant phenotype is mediated by two early gene products, E6 and E7 [9]. Both viral proteins interact with products of cellular human tumor-suppressor genes [9-11]: the E6 protein can bind and promote degradation of cell-encoded p53, and the E7 protein interacts with the retinoblastoma susceptibility gene product. Expression of E6 and E7 proteins has been shown to be necessary and sufficient for HPV-16 transformation of human cells [12-14]. Therefore, the E6 and E7 proteins are potential molecular targets for new preventive and therapeutic modalities. Our group has developed a vaccine that uses antigens from the E6 protein since the T-cell immune responses to the HPV-16 E6 protein are associated with HPV clearance and SIL regression. Therefore, therapeutic vaccines targeting E6 and E7 may have potential to control HPV-associated malignancies.

Positive results from experimental vaccination systems in animal models have led to several prophylactic and therapeutic vaccine clinical trials. These investigations include administering these proteins in live vectors, peptides or protein, nucleic acid form, as components of chimeric VLPs, or as cell-based vaccines. These vaccines may prove invaluable for the prevention of HPV infection and life threating cancers these infections may bring. Although numerous preclinical and clinical trials have evaluated prophylactic HPV

vaccines during the past few decades, these vaccines do not help those who already have established HPV infections [15]. Clinical trials have demonstrated excellent vaccine efficacy in women negative for HPV-16 or HPV 18 [16, 17], but the duration of protection remains to be determined, and a study of the bivalent vaccine showed no evidence of enhanced viral clearance in women with preexisting HPV infections. Therefore, therapeutic vaccines are needed for cases in which HPV infection is already established and in which HPV-related diseases have already developed, particularly because the prophylactic vaccine coverage rate in the targeted group (girls, ages 13-17 years) reportedly is low nationally [18]. Our group is developing PepCan as an HPV therapeutic vaccine. It consists of four current good manufacturing practice (cGMP)-grade synthetic peptides covering the E6 protein of HPV type 16 (HPV-16), along with Candin® a skin testing antigen derived from Candida albicans (Nielsen BioSciences, San Diego, CA) as a novel vaccine adjuvant. The immune response to the E6 protein has been associated with good clinical outcomes [19-21].

The safety of the HPV peptide-Candin® combination was examined in mice in a multiple-dose toxicity study at Southern Research Institute (Birmingham, AL). The 25 μg and 50 μg per peptide doses were used, which were 25 times the human equivalent to the 250 μg and 500 μg per peptide doses when adjusted for body-surface area. Because four administrations 3 weeks apart were planned for the Phase I trial, we performed five administrations in the animal study, as recommended, but with a greater frequency (weekly inoculations on days 1, 8, 15, 22, and 29). The dorsal side of each animal was divided into four areas, and on each dosing day, the animals received intradermal injections (100 μL), which were split approximately equally between two sites (areas 1 and 2 on days 1, 15, and 29; areas 3 and 4 on days 8 and 22).

Table 1. Six groups of C57BL/6 female mice examined in the multiple-dose toxicology study

Group	Treatment	Antigens Dose (μg/mouse)	Adjuvant Dose (μL/mouse)	Total Vol. (μL/mouse)	Animals (*n*)	
					Day 32 Core*	Day 60 Recovery†
1	Vehicle control	0	0	100	10 F	10 F
2	Adjuvant alone	0	30	100	10 F	10 F
3	Antigens alone	25	0	100	10 F	10 F
4	Antigens alone	50	0	100	10 F	10 F
5	Antigens + adjuvant	25	30	100	10 F	10 F
6	Antigens + adjuvant	50	30	100	10 F	10 F

**Core, to be sacrificed 3 days after injection. †Recovery, to be sacrificed 4 weeks after the last injection. F, female.*

All mice in all groups survived to scheduled necropsy (Table 1). Administration of vehicle, Candin®, low- or high-dose peptides without Candin®, or low- or high-dose peptides with Candin® had no effect on body weights, body temperatures, food consumption, or absolute or relative organ weights of mice. Clinical signs associated with treatment included scabs and sore/ulcer at the dosing sites; these signs appeared shortly after dosing and resolved within a few days of appearance. Scabs and sore/ulcer at the dosing sites appeared sporadically in all groups (including the vehicle control) with no dose response in incidence and were therefore considered to have been due to the treatment procedure itself rather than to the peptides, Candin®, or the combination. The only change in clinical pathology parameters that was considered to be potentially related to administration of peptide and adjuvant was a statistically significant but minimal elevation in the mean eosinophil count that was observed on day 32 for mice treated with 50 μg of each of the peptides with Candin®, compared to the mean value for mice in the vehicle control group. This finding was transient and was not reported on day 60. Microscopic observations on day 32 of test article-

related lesions were found in mice that received any formulations containing peptides (Groups 3–6) and included chronic-active inflammation (infiltrations of neutrophils, eosinophils, and mononuclear cells). The findings on day 60 were more chronic; primarily mononuclear cells were seen with scattered neutrophils and eosinophils. The only observed test article-related macroscopic lesion was a crust on day 32 at the cranial injection site of one animal treated with 50 µg of each peptide without Candin® and of one animal treated with 50 µg of each peptide with Candin®. In conclusion, the only toxicity observed was a transient, minimal eosinophil elevation in animals receiving 50 µg of peptides with Candin® compared to the vehicle control; this was accompanied by injection-site inflammation (including eosinophil infiltration) on day 32.

PepCan was first tested in human to treat biopsy-proven high-grade squamous intraepithelial lesions (HSILs) in a Phase I single-arm, single-site study (NCT01653249). Subjects (n=52) were enrolled between September 2012 and January 2015, and those with biopsy-proven cervical intraepithelial neoplasia (CIN) 2/3 (n=34) (synonymous to HSIL) were eligible for vaccination. The initial part of the clinical trial was dose-escalation in style in which six subjects each were consecutively assigned to increasing amounts of HPV-16 E6 peptides (50 µg/peptide, 100 µg/peptide, 250 µg/peptide, and 500 µg/peptide). The amount of Candin® was constant at 300 µL. After the dose escalation phase, an additional 10 subjects were vaccinated at the optimal dose of 50 µg/peptide as determined by histological regression. At the screening visit, the cervix was visualized under a colposcope after applying acetic acid, biopsies were obtained, Thin-Prep (Hologic, Marlborough, MA) was collected for HPV-DNA testing (Linear Array HPV Genotyping Test, Roche Molecular Diagnostics, Pleasanton, CA), and routine laboratory testing was performed (complete blood count, sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, aspartate transaminase, alanine transaminase, lactate dehydrogenase, γ-glutamyl transpeptidase, total bilirubin, and direct bilirubin). Subjects who already were diagnosed with biopsy-proven CIN2/3 were also eligible as long as the first vaccine injection could be given within 60 days, and other inclusion criteria were met (ages 18 to 50 years old, blood pressure ≤ 200/120 mm Hg, heart rate 50 to 120 beats per minute, respiration ≤ 25 breaths per minute, temperature ≤ 100.4°F, white count ≥ 3 x 109/L, hemoglobin ≥ 8 g/dL, and platelet count ≥ 50 x 109/L). Being positive for HPV-16 was not required due to possible cross-protection [19, 20, 22, 23] and de novo immune stimulation [24, 25]. Exclusion criteria included a history of disease or treatment causing immunosuppression, pregnancy, breast-feeding, allergy to Candida, a history of severe asthma, current use of beta-blocker, and a history of invasive squamous cell carcinoma of the cervix. Urine pregnancy test was performed prior to each injection, and blood was drawn for routine laboratory testing and immunological assessments immediately prior to the first and third injections. The vaccine was administered intradermally in any limb. Twelve weeks after the last injection, blood was drawn, ThinPrep sample was collected and LEEP was performed. The study gynecologists described the presence of visible lesions and their locations. Digital photographs were taken as additional documentation. Pre-vaccination biopsies from the screening visits and post-vaccination LEEP biopsies were read by the staff pathologists at the study institution. In addition, the study pathologist reviewed all slides of all LEEP samples. The highest grade of pathology found in any lesion was used to determine eligibility to be vaccinated and to assess histological response. Safety and tolerability were assessed from the time informed consent was obtained until the day LEEP was performed using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 4.0. Dose-limiting toxicities were defined as vaccine-related allergic and autoimmune AEs greater than grade 1 and any other AEs greater than grade 2. Efficacy was based on histological grading of the LEEP samples. A subject with no dysplasia or cervical intraepithelial neoplasia 1 (CIN 1) was considered to be a complete responder, and a subject with CIN2/3 measuring ≤ 0.2 mm2 was considered to be a partial responder (ScanScope® CS and

ImageScope™ software, Aperio, Vista, CA). When responders and non-responders were compared, complete and partial responders were combined.

One hundred thirty-two injections have been given to 34 subjects. No dose limiting toxicities were reported. The most common AEs were immediate and delayed injection-site reactions. Although delayed injection-site reaction was defined as occurring at or more than 24 hours after injection, it was not uncommon for it to appear a few to several days after injection. More grade 2 immediate injection-site reactions but not delayed injection-site reactions were recorded at the higher two doses compared to the lower two doses (odds ratio of 6.3 [1.98, 20.3], p<0.0001, for the immediate reaction; and 2.0 [0.6, 7.1], p=0.3, for the delayed reaction). Other vaccine-related or possibly vaccine-related AEs, which occurred with ≥ 5% of injections, in the order of decreasing number of occurrences, were myalgia, headache, nausea, fatigue, hypokalemia, fever, and flu-like symptoms. None of these AEs was greater than grade 2.

The histological response rates in order of increasing doses were 50%, 50%, 33%, and 40%. The overall histological response rate was 45%, and none progressed to cervical squamous cell carcinoma. In comparison, a historical placebo group in another clinical trial of HPV therapeutic vaccine with a similar study reported a regression rate of 22% [26]. No statistically significant differences were detected when histological response rates were compared (1) between subjects with entry diagnosis of CIN 2 versus CIN3, (2) between subjects ≤ 25 years of age versus > 25 years of age, and (3) between subjects who were HPV-16-positive versus those who were not. The mean number of cervical quadrants with visible lesions decreased significantly from 1.9 quadrants to 0.8 quadrants after vaccination (p=0.001).

The number of HPV types detected prior to vaccination ranged from zero to six types. The rate of at least one HPV type becoming undetectable at exit was the highest (85%) at the 50 µg dose. When the HPV types, which became undetectable, were grouped into HPV-16, HPV-16-related, other high-risk types, and low-risk types, the rate of undetectability was paradoxically higher for non-HPV-16 types. Of 13 subjects in whom HPV-16 DNA was detected prior to vaccination, it became undetectable in 3 subjects and was persistent in 9 subjects. One HPV-16-positive subject did not complete the study. In the subjects with persistent HPV-16 viral loads, a significant decrease (mean of 840 copies per cells to 76.6 copies per cell, p=0.008) was observed in 8 of the 9 subjects (Figure 1).

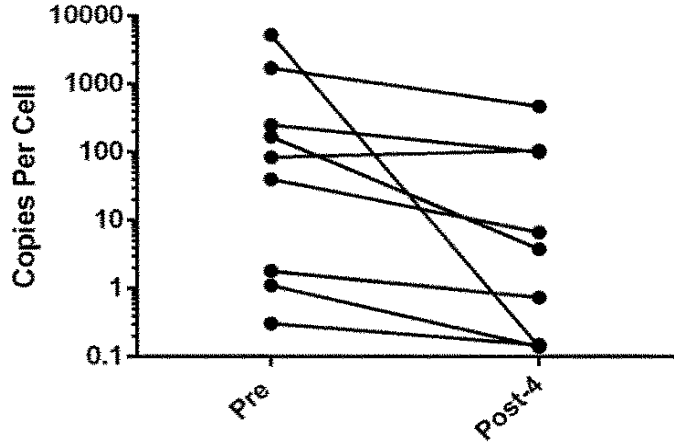


Figure 1: *A significantly decreased HPV-16 viral loads of subjects who were positive for HPV-16 at entry and exit. Of 13 subjects in whom HPV-16 DNA was detected prior to vaccination, it became undetectable in 3 subjects and was persistent in 9 subjects. One HPV-16-positive subject did not complete the study. In the subjects with persistent HPV-16 viral loads, a significant decrease (mean of 840 copies per cells to 76.6 copies per cell, p=0.008) was observed in 8 of the 9 subjects.*

Immunological responses to HPV-16 E6, as measured by IFN-γ ELISPOT assay, were similar among the first 3 dose levels in terms of detecting positive response to at least one new E6 region and for the increase in response being statistically significant. The lowest response rate was observed for the 500 µg dose level. No immunodominant HPV-16 E6 region was identified and the number of regions to which new immune responses were detected was also variable. Immune profiling (Figure 2, upper panel) showed statistically significant increases in circulating T-helper type 1 (Th1) cells after 2 (p=0.02) and 4 vaccinations (p=0.0004). T-helper type 2 (Th2) cells initially increased significantly (p=0.01) but decreased to below the baseline level after 4 vaccinations, although not significantly. Regulatory T-cell (Treg) levels were minimally changed. The differences in Treg levels pre-vaccination (p=0.03) and post-2 vaccinations (p=0.04) between these two groups were statistically significant (Figure 2, lower panel).

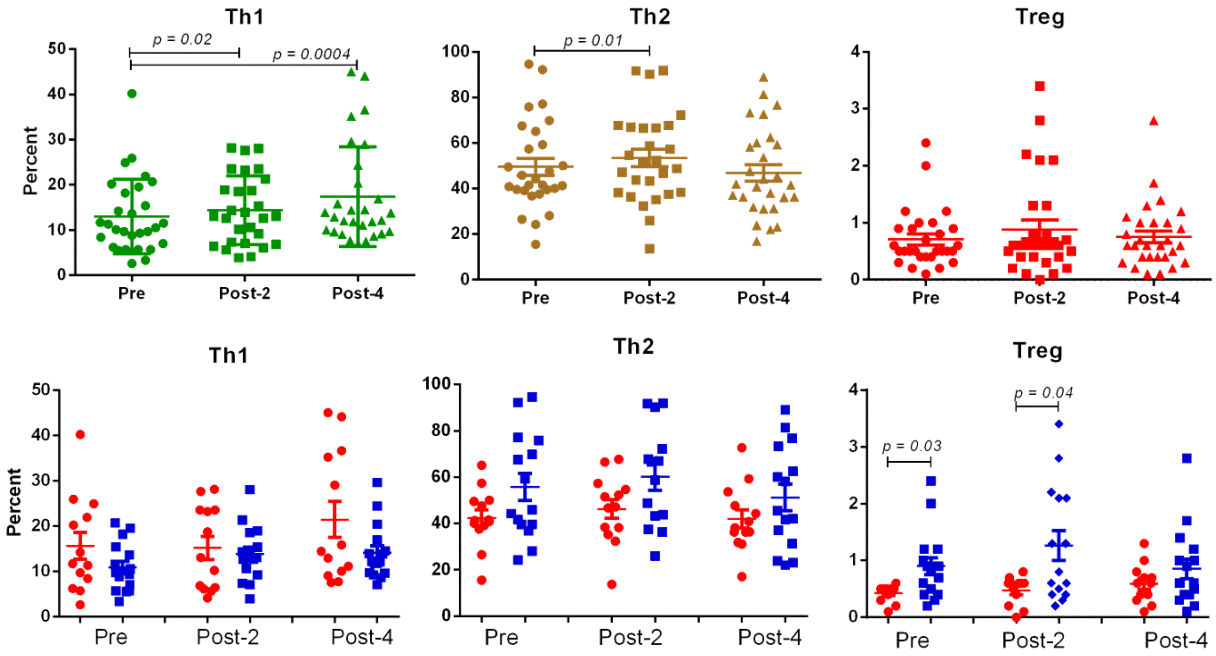


Figure 2: *FACS analysis of peripheral immune cells. Upper panel: Systemic Th1, Th2, and Treg before, after 2, and after 4 vaccinations. Lower panel: Responders are indicated by filled circles and non-responders by filled squares. None of the subjects with pre-vaccination Treg levels ≥0.7% was a vaccine responder. Cells were first stained with antibodies for surface markers CD3, CD4, and CD25. Staining for intracellular T-bet, GATA3, and Foxp3 was performed with the Foxp3 staining kit according to the manufacturer's instructions (eBioscience). Th1 cells were expressed as a percentage of CD4 cells positive for T-bet, Th2 cells as a percentage of CD4 cells positive for GATA3, and Tregs as a percentage of CD4 cells positive for CD25 and Foxp3. Paired t-test (upper panel) or Wilcoxon rank-sum test (lower panel) was used.*

A follow-up clinical trial, "A Phase II Clinical Trial of PepCan Randomized and Double-Blinded to Two Therapy Arms for Treating Cervical High-Grade Squamous Intraepithelial Lesions", is ongoing in which eligible subjects are treated with PepCan or Candin® to prevent progression to cervical cancer (NCT02481414). Instead of performing LEEP at 3 months, the subjects are being observed for 12 months. Four quadrant cervical biopsies are being performed to assess histological responses. No serious toxicity has been reported to date.

The bacterial inhabitants of the human gut constitute an enormous and dynamic ecosystem. The diversity of gut microbiome has been shown to correlate not only with higher number of Tregs leading to reduced susceptibility of allergic disorders but also with better response to cancer immunotherapy in mice and human [27-30]. Emerging studies have revealed the importance of the gut microbiome in shaping host immune responses through the modulation of Tregs. Constitutive deficiency in the expression of transcription factor forkhead box P3 (Foxp3) and thus Tregs function results in high levels of serum immunoglobulin E in mice and human [31, 32], which leads to the susceptibility to allergic disorders. Accumulating evidence indicates that specific microbial species induce colonic Treg generation in mice [33, 34]. The role of the microbiome in cancer immunotherapy has been studied in mice and humans. A recent study in a murine model reveals that the antitumor effects of cytotoxic T-lymphocyte associated protein 4 is a major negative regulator of T cell activation. Its blockade depends on distinct Bacteroides species [29]. A significant difference in the diversity and composition of the gut microbiome in responders versus non-responders to anti-PD-1 (anti-programmed death-1) agents of patients with metastatic melanoma has been reported [27]. Patients who responded to anti-PD-1 agents had more diversity of gut bacteria and a predominance of the Ruminococcaceae family compared to patients who did not respond to PD-1 inhibition [27].

2.2 STUDY RATIONALE

This current study has been designed to evaluate the safety and efficacy of giving seven injections of PepCan or placebo over approximately a 24-month period in subjects with head and neck cancers who achieved remission. PepCan may prove to be beneficial in treating many stages of HPV-related malignancies starting from infection to cancer. Safety, efficacy in terms of reduced cancer recurrence, immunological responses and profiles, and gut microbiome changes will be assessed.

2.3 SCIENTIFIC RATIONALE

Rationale for Proposed Route of Administration, Dose, and Regimen

The intradermal route of administration was proposed to make use of Langerhans cells as antigen-presenting cells. A Phase I clinical trial of a peptide vaccine for prostate cancer administered through this route has shown promising immunogenicity [35]. Extremities have been chosen as the site of administration because of the ease of access, as well as availability of sufficient data demonstrating efficacy of HPV peptides delivered at these sites.

A phase I dose-escalation clinical trial in cervical cancer with PepCan showed no dose-limiting toxicities. The most common AEs were injection-site reaction [36, 37].

After showing the safety of PepCan in Phase I, Nakagawa et al. also showed from the six subjects enrolled at each dose level (50, 100, 250, and 500 µg per peptide) the best histological and virological response was found at the 50µg dose with a regression rate of 50% HPV clearance rate of at least one type at 85% [36]. The study also shows systemic Th1 cells were significantly increased after the injection regimen and an HPV type 16 viral load decrease. Additionally, this increase suggests Candin®, which induces interleukin-12 (IL-12) in vitro, may have a promoting effect on the Th1 cells as it has shown to induce IL-12 production by Langerhans cells *in vitro* [38, 39].

In the Phase I clinical trial of PepCan treating women with biopsy-proven high-grade squamous intraepithelial neoplasia (HSIL), four injections were given with 3 week intervals [36, 37]. Three-week intervals have been shown to be better than weekly intervals in terms of immune responses detected [40]. PepCan is designed to work by stimulating immune response to HPV antigens and by shifting the type of immune response to Th1 response, which is known to fight infection and cancer. While the HPV peptides are likely to be responsible for stimulating immune response to HPV antigens, Candin®, the adjuvant, is likely to be responsible for the Th1 shift. What is not known is how long this Th1 shift lasts as the pre-cancer and cancer are known to create immunosuppressive environments.

Therefore, in this Phase I/II clinical trial of PepCan in subjects with head and neck cancer who achieved remission, the subjects in the vaccination arm will be given 7 doses of PepCan, so the duration of Th1 shift will be prolonged. The main goal would be to assess the safety and efficacy of giving 7 total doses of PepCan. For the assessment of efficacy, a randomized double-blind placebo-controlled design will be used in which the subjects will receive PepCan versus placebo at a 3:1 ratio. The first 4 doses will be given every 3 weeks, and the remaining 3 doses will be given every 3 months.

2.4 RISK/BENEFIT ASSESSMENT

Potential Risks

Risks of HPV Peptides

No dose-limiting toxicities were observed in the Phase I trial. However, the total number of individuals who have received the vaccine is small, and there may be relatively rare complications that have not surfaced. The most common AEs were injection-site reactions, and none of the subjects experienced dose-limiting toxicities [36, 37]. Most commonly reported AEs were local swelling, redness, increased skin temperature, and local pain at injection sites. None of the vaccine-related events exceeded grade 2 according to the NCI CTCAE Version 4.0.

Risks of Candin®

Although the Candin® antigen is FDA-approved as a skin-test antigen for human use, the HPV peptide-Candin® (PepCan) combination has not been approved. According to the Candin® package insert, local reactions have included swelling, pruritus, and vesiculation. Immediate hypersensitivity reaction to Candin® has occurred in some individuals. Such reactions are characterized by edematous hives surrounded by a zone of erythema. They occur 15 to 20 minutes after intradermal injection. The safety of Candin® when used at a higher dose with increased frequency (up to 10 injections per subject) has also been demonstrated by the Phase I clinical trial of wart treatment we recently concluded [25]. The most common AEs were injection-site reaction with local swelling, redness, increased skin temperature, and local pain.

Risks of Combining HPV Peptides and Candin®

The safety of injecting the HPV-16 E6 peptides combined with Candin® was tested in the Phase I clinical trial of a human papillomavirus and appears to be safe [36, 37]. In the Phase I trial, subjects received a series of 4 doses of PepCan. Therefore, we do not anticipate any serious side effects when the number of injections is increased to 7 from 4.

Strategies to Minimize Risks

A pregnancy test will be performed prior to each vaccination in women of childbearing potential. All subjects will be asked to stay in the clinic for a minimum of 30 min after each injection to assure that immediate hypersensitivity reactions, if present, will be identified and treated appropriately. To ameliorate possible and anticipated localized minor vaccination site reactions, subjects will be offered a dose of ibuprofen or naproxen after a minimum of 30 min observation period.

Potential Benefits

The intended benefit is the development of a novel HPV therapeutic vaccine regimen to reduce cancer recurrence for patients with previously diagnosed head and neck cancer. This study will contribute to this goal by assuring the safety and effectiveness of HPV peptides in PepCan.

3. OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
PRIMARY		
Determine the safety of a 7-injection scheduling of PepCan regimen.	Careful monitoring of adverse events (AEs) at all ten (10) visits (2 years) based on NCI CTCAE Version 5.0.	Investigators will use NCI CTCAE Version 5.0 to assess AEs and evaluate the safety of PepCan.
SECONDARY		
Determine the efficacy of a 7-injection PepCan regimen.	The absence of recurrence will be assessed at all ten (10) visits (2 years). Recurrence rates will be compared between the PepCan and placebo arms.	The goal of PepCan vaccine is to reduce recurrence of head and neck cancer.
TERTIARY		
Determine immunological responses to a 7-injection PepCan regimen.	T-cell (ELISPOT assay) and immune regulatory cell responses (FACS analysis) at Visits 1, 5, 8, and 9 will be assessed before and after vaccination.	Immunological responses are important in preventing cancer recurrence.
EXPLORATORY		
Compare the profile of gut and oral microbiome before and after PepCan regimen.	DNA sequencing methods will be utilized. Stool samples will be analyzed after Screening Visit, and Visits 5, 7, and 8. Oral wash samples will be analyzed at Screening Visit, and Visits 5, 7, and 8.	Diversity of gut microbiome is now recognized to be associated with favorable response to cancer immunotherapy [27].

4. STUDY DESIGN

4.1 OVERALL DESIGN

This is a Phase I/II randomized, double-blind, placebo controlled, multi-site study of PepCan. It is designed to show the safety and efficacy of a 7-dose regimen of PepCan over a two-year period in terms of reducing cancer recurrence rate by comparing the recurrence rates between the PepCan and the placebo arm. The ratio of the number of subjects who will receive PepCan versus placebo will be 3:1. The study randomization schematic will be constructed by the Study Statistician. Subjects will be randomized in a 3:1 ratio to PepCan or placebo, and randomization will be done in a book form by Research Pharmacy. Up to 150 subjects will be for screened until 100 subjects are eligible for injection.

4.2 END OF STUDY DEFINITION

The end of study is defined as the time that all data from all participating sites have been collected, reviewed, and analyzed.

5. STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Able to provide informed consent
2. Male or female 18 years of age or older
3. Squamous cell carcinoma of the head and neck who have completed curative therapy (surgery and/or radiation and/or chemotherapy) within the previous 120 days
4. Performance status of ECOG 0-2
5. No Evidence of Disease (NED) based on clinical and/or radiographic evaluations
6. Vital Signs recorded
   a. Blood pressure (BP) (<200/120mm Hg)
   b. Heart rate (50-120 beats per min)
   c. Respiratory rate (<25 breaths per min)
   d. Temperature (<100.4°F)
7. Blood work done at Screening Visit
   a. White count (≥ $3x10^9$/L)
   b. Hemoglobin (≥ 7 g/dL)
8. Willing and able to comply with the requirements of the protocol

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Positive urine pregnancy test for women of childbearing potential
2. Being pregnant or attempting to be pregnant with the period of study participation
3. Women who are breast feeding or plan to breast feed within the period of study participation
4. Patients who are allergic to Candin® or yeast
5. History of severe asthma requiring emergency room visit or hospitalization within the past 5 years
6. Patients who have previously received PepCan
7. History of recurrence of squamous cell carcinoma of the head and neck
8. If in the opinion of the PIs or other Investigators, it is not in the best interest of the patient to enter or continue in this study

5.3 SCREEN FAILURES

Screen failures are defined as subjects who were consented to participate in the clinical trial but are not eligible after screening for enrollment in the study intervention.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

- Recruitment will take place at each site/institution that is participating in this multi-site study.
- Investigators at sites will approach their own patients whom they feel are potentially eligible for the study. The clinical research team at sites will also review medical records to find eligible patients. After discussing the study with the patient, the patient will have the opportunity to review and discuss the informed consent form with the clinical research nurse to decide if they would like to participate.
- Historically under-represented populations will be encouraged to participate in order to meet target sample size and conform to the NIH Policy on Inclusion of Women and Minorities as Participants in Research Involving Human Subjects.

5.5 RECURRENCE

If a subject has clinical and/or radiological evidence of recurrence and a study physician determines the subject to be in recurrence, the subject will not receive further study interventions. The subject will be included in the recurrence group, and will complete participation by filling out the “Recurrence Questionnaire”.

6. INVESTIGATIONAL PRODUCT

6.1 TEST ARTICLE

HPV Peptides

PepCan consists of four HPV-16 E6 peptides:

E6 1-45 (Ac-MHQKRTAMFQDPQERPRKLPQLCTELQTTIHDIILECVYCKQQLL-NH2)

E6 46-80 (Ac-RREVYDFAFRDLCIVYRDGNPYAVCDKCLKFYSKI-NH2)
E6 81-115 (Ac-SEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINCQK-NH2)
E6 116-158 (Ac-PLCPEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRETQL-NH2)
(US Patent No. 8,652,482)

The four peptides will be provided in a single vial in lyophilized form at the 50 µg/peptide/dose, and will be stored at -80°C (+10°C) until ready to use. The peptides are not approved for multi-dosing, and would need to be used on the day of reconstitution.

The UAMS Research Pharmacy will be responsible for providing the peptide and training all participating sites on peptide receipt, storage and preparation of the peptide prior to vaccination visits. The pharmacy manual provided by the study Sponsor will also have information pertaining to this.

CANDIN®

Candida Albicans Skin Test Antigen for Cellular Hypersensitivity will be supplied in the commercially marketed drug Candin®. The vials will be stored at 2°C to 8°C as directed by the package insert until use. This product is approved for multi-dosing. The dose of Candin® per injections for this study is 0.3 mL.

COMBINING HPV PEPTIDES AND CANDIN®

The cGMP-grade peptides were produced by CPC Scientific (San Jose, CA). They were formulated, vialed, and lyophilized at 550 µg per peptide by Integrity Bio, Inc. (Camarillo, CA), and were designed to be reconstituted with 0.77 mL of sterile water per vial. After adding Candin®, the mixture will be mixed lightly prior to inoculation. There are four cGMP-grade synthetic peptides covering the entire length of the HPV-16 E6 protein in PepCan. The E6 protein was divided roughly in four parts preserving the most immunogenic areas (E6 46-70 and E6 91-115) [20].

On the day of, use sterile water will be added to a vial containing the four cGMP peptides. Reconstituted peptides will be drawn in a syringe depending on the dose level and 0.3 mL of Candin® will be drawn into the same syringe. The combined peptide-Candin® mixture should be kept on ice or in refrigerator until immediately before injection.

PLACEBO

The placebo will be sterile 0.9% Normal Saline for injection. The UAMS Research Pharmacy will be responsible for providing the placebo and training all participating sites on receipt, storage and preparation of the placebo prior to vaccination visits. The pharmacy manual provided by the study sponsor will have information pertaining to this.

TEMPERATURE LOGS

Daily temperature logs will be maintained by the local site's Pharmacy per standard operating procedures of the Pharmacy. Any deviations in temperature range should be reported to the UAMS Office of Research Regulatory Affairs (ORRA) and the UAMS PI.

Drug Accountability Records

Drug accountability records will be maintained per Pharmacy, Institutional, FDA, NIH and other applicable policies.

6.2 TREATMENT REGIMEN

The study medication is a vaccine regimen consisting of seven PepCan or placebo injections. The route of administration is intradermal injection at subject's limbs at a 50 µg/peptide/injection. The schedule is 1 injection every three weeks for the first 4 injections, and then one injection every 3 months until a total of 7 injections has been given.

7. STUDY ASSESSMENTS AND PROCEDURES

7.1 STUDY VISITS

Screening Visit

- Study staff may contact potentially eligible subject prior to screening visit to provide study overview
- Review inclusion/exclusion criteria
- Obtain informed consent (if not previously obtained)
- Complete Demographic and Contact Information Form
- Ask the subject to complete the Screening Visit Questionnaire
- Obtain subject's history (may be performed within 30 days prior to subject signing consent)
  - Medical history
    - Specifically review of past cancer diagnosis, treatment, remission
    - Note specific tests, including performance evaluation, ENT evaluation and/or radiography, to support No Evidence of Disease (NED) status
    - Note p16 and/or HPV status of tumor if available
  - Drug allergies
  - Concomitant medications
- Perform a physical examination of head and neck including the vital signs which would include blood pressure, heart rate, respiratory rate, and temperature (may be performed within 30 days prior to the subject signing consent)
  - For screening vital signs outside of the acceptable ranges
  - they can be repeated within 30 days of signing informed consent

  OR
  - subject can be enrolled if the Medical Monitor determines it is safe
- For male subjects:
  - Inform males that the anticipated risks of the vaccine to sexual partners are minimal. Symptoms (see section 2.4 and also informed consent form risks and discomforts) experienced by vaccine recipients are temporary and are not likely to transfer to sexual partners or to household contacts. Therefore, birth control measures for female sexual partners are not required.
- For women of child-bearing potential:
  - Discuss the risks involved in becoming pregnant while receiving vaccine
  - Ask which birth-control method will be used while participating in the vaccine trial (FDA acceptable forms of preventing pregnancy include oral contraceptives, contraceptive

patches/rings/implants/shots, double-barrier methods (e.g. condoms and spermicide), abstinence and/or vasectomies of a male partner with a documented second acceptable method of birth control.
    - Urine Pregnancy Test for women of child-bearing potential
- Collect blood samples (standard of care clinical blood tests may be used if performed within 30 days prior to the subject signing consent (i.e. study enrollment.))
    - CBC with diff testing
        - White count (≥ $3x10^9$/L acceptable)
        - Hemoglobin (≥ 7 g/dL acceptable)
        - Hematocrit (no restriction)
        - Platelet count (no restriction)
    - Chem 7 and LFT testing
        - Calcium (no restriction)
        - Sodium (no restriction)
        - Potassium (no restriction)
        - CO_2 (no restriction)
        - Chloride (no restriction)
        - BUN (no restriction)
        - Creatinine (no restriction)
        - Lactate dehydrogenase (no restriction)
        - Alkaline phosphatase (no restriction)
        - Alanine amino transferase (no restriction)
        - Aspartate amino transferase (no restriction)
        - Total Bilirubin (no restriction)
- Collect the oral wash sample
    - Provide subject with a cup with 10mL of mouthwash
    - Ask subject to swish vigorously for at least 30 seconds then spit back into the same cup (no brushing for 120 minutes and eating for 10 minutes before collection)
    - Ask the subject to complete the oral wash antibiotic usage questionnaire
- Distribute stool collection kit to subject with instructions, including the stool sample antibiotic usage questionnaire to be completed on the days of stool collection.
    - For subjects who leave prior to learning whether they are eligible or not, study staff should contact the subject and inform them of their eligibility status.
    - Ineligible subjects should be informed that they do not need to provide a stool sample and that they may discard the kit.
    - Visit 1 should be scheduled after the stool sample is received. It must be scheduled within 90 days of the screening visit.

VISIT 1 (AFTER RECEIVING THE STOOL SAMPLE BACK (WITHIN 90 DAYS OF SCREENING VISIT))

- Ask if any medications have been started or stopped since the last visit
- Urine pregnancy test prior to vaccination for women of child-bearing potential
- Take vital signs prior to injection
- Blood will be drawn for
    - Immunomonitoring and other analyses (eight 10.0 mL rubber green top sodium heparin tubes)
    - CBC with diff
    - Chem 7 and LFTs

- Administer vaccination injection
- Monitor for any immediate adverse reactions for at least 30 minutes.
- After a minimum of 30 minutes have passed since injection, retake vitals.
- Offer dose of ibuprofen or naproxen (optional to take)

VISIT 2 (3 WEEKS AFTER VISIT #1 ± 7 DAYS)

- Ask the subjects to verbally report if they have experienced any side effects since the last injection.
- Ask the subjects to verbally report if any medications have been started or stopped since last visit.
- Urine pregnancy test prior to vaccination for women of child-bearing potential
- Take vital signs prior to injection
- Administer vaccination injection
- Monitor for any immediate adverse reactions for at least 30 minutes.
- After a minimum of 30 minutes have passed since injection, retake vitals.
- Offer dose of ibuprofen or naproxen (optional to take)

VISIT 3 (3 WEEKS AFTER VISIT #2 ± 7 DAYS)

- Ask the subjects to verbally report if they have experienced any side effects since the last injection.
- Ask the subjects to verbally report if any medications have been started or stopped since last visit.
- Urine pregnancy test prior to vaccination for women of child-bearing potential
- Take vital signs prior to injection
- Blood will be drawn for
  - CBC with diff
  - Chem 7 and LFTs
- Administer vaccination injection
- Monitor for any immediate adverse reactions for at least 30 minutes.
- After a minimum of 30 minutes have passed since injection, retake vitals.
- Offer dose of ibuprofen or naproxen (optional to take)

VISIT 4 (3 WEEKS AFTER VISIT #3 ± 7 DAYS)

- Ask the subjects to verbally report if they have experienced any side effects since the last injection.
- Ask the subjects to verbally report if any medications have been started or stopped since last visit.
- Urine pregnancy test prior to vaccination for women of child-bearing potential
- Take vital signs prior to injection
- Administer vaccination injection
- Monitor for any immediate adverse reactions for at least 30 minutes.
- After a minimum of 30 minutes have passed since injection, retake vitals.
- Offer dose of ibuprofen or naproxen (optional to take)

VISIT 5 (3 MONTHS AFTER VISIT #4 ± 14 DAYS)

- Ask the subjects to verbally report if they have experienced any side effects since the last injection.
- Ask the subjects to verbally report if any medications have been started or stopped since last visit.
- Urine pregnancy test prior to vaccination for women of child-bearing potential
- Collect oral wash sample
  - Provide subject with a cup with 10mL of mouthwash

        - Ask subject to swish vigorously for at least 30 seconds then spit back into the same cup (no brushing for 120 minutes and eating for 10 minutes before collection)
        - Ask the subject to complete the oral wash antibiotic usage questionnaire
- Perform a physical examination per standard of care
- Take vital signs prior to injection
- Blood will be drawn for
    - Immunomonitoring and other analyses (eight 10.0 mL rubber green top sodium heparin tubes)
    - CBC with diff
    - Chem 7 and LFTs
- Administer vaccination injection
- Monitor for any immediate adverse reactions for at least 30 minutes.
- After a minimum of 30 minutes have passed since injection, retake vitals.
- Offer dose of ibuprofen or naproxen (optional to take).
- Distribute stool collection kit to subject with instructions, including the stool sample antibiotic usage questionnaire to be completed on the days of stool collection.

VISIT 6 (3 MONTHS AFTER VISIT #5 ±14 DAYS)

- Ask the subjects to verbally report if they have experienced any side effects since the last injection.
- Ask the subjects to verbally report if any medications have been started or stopped since last visit.
- Urine pregnancy test prior to vaccination for women of child-bearing potential
- Perform a physical examination per standard of care
- Take vital signs prior to injection
- Blood will be drawn for
    - CBC with diff
    - Chem 7 and LFTs
- Administer vaccination injection.
- Monitor for any immediate adverse reactions for at least 30 minutes.
- After a minimum of 30 minutes have passed since injection, retake vitals.
- Offer dose of ibuprofen or naproxen (optional to take).

VISIT 7 (3 MONTHS AFTER VISIT #6 ± 14 DAYS)

- Ask the subjects to verbally report if they have experienced any side effects since the last injection.
- Ask the subjects to verbally report if any medications have been started or stopped since last visit.
- Urine pregnancy test prior to vaccination, for women of child-bearing potential
- Collect oral wash sample
    - Provide subject with a cup with 10mL of mouthwash
        - Ask subject to swish vigorously for at least 30 seconds then spit back into the same cup (no brushing for 120 minutes and eating for 10 minutes before collection)
        - Ask the subject to complete the oral wash antibiotic usage questionnaire
- Perform a physical examination per standard of care
- Take vital signs prior to injection
- Blood will be drawn for
    - CBC with diff
    - Chem 7 and LFTs
- Administer vaccination injection.

- Monitor for any immediate adverse reactions for at least 30 minutes.
- After a minimum of 30 minutes have passed since injection, retake vitals.
- Offer dose of ibuprofen or naproxen (optional to take).
- Distribute stool collection kit to subject with instructions, including the stool sample antibiotic usage questionnaire to be completed on the days of stool collection.

VISIT 8 (6 MONTHS AFTER VISIT #7 ± 30 DAYS)

- Ask the subjects to verbally report if they have experienced any side effects since the last injection.
- Ask the subjects to verbally report if any medications have been started or stopped since last visit.
- Urine pregnancy test for women of child-bearing potential
- Collect oral wash sample
  - Provide subject with a cup with 10mL of mouthwash
    - Ask subject to swish vigorously for at least 30 seconds then spit back into the same cup (no brushing for 120 minutes and eating for 10 minutes before collection)
    - Ask the subject to complete the oral wash antibiotic usage questionnaire
- Perform a physical examination per standard of care
- Take vital signs
- Blood will be drawn for
  - Immunomonitoring and other analyses (eight 10.0 mL rubber green top sodium heparin tubes)
  - CBC with diff
  - Chem 7 and LFTs
- Distribute stool collection kit to subject with instructions, including the stool sample antibiotic usage questionnaire to be completed on the days of stool collection.

VISIT 9 (6 MONTHS AFTER VISIT #8 ± 30 DAYS)

- Ask the subjects to verbally report if they have experienced any side effects since the last injection.
- Ask the subjects to verbally report if any medications have been started or stopped since last visit.
- Ask the subject to complete the Visit 9 Questionnaire
- Urine pregnancy test for women of child-bearing potential
- Perform a physical examination per standard of care
- Take vital signs
- Blood will be drawn for
  - Immunomonitoring and other analyses (eight 10.0 mL rubber green top sodium heparin tubes)
  - CBC with diff
  - Chem 7 and LFTs

Optional Follow Up Visit

- A follow-up visit may be scheduled after the screening visit, after the vaccination visits, and at any time during study participation to receive follow-up instructions, be evaluated for AEs, or address study-related concerns as needed.

7.2 SAFETY ASSESSMENTS

As one of the primary endpoints of this study is safety, the monitoring of AEs will be diligently recorded and reviewed. In the clinical setting, subjects will report any AEs since their last visit and immediately following each vaccination. Additionally, vital signs will be taken pre- and post-vaccination to monitor for

fluctuations in these parameters. Vital signs will be taken and blood drawn (CBC with diff, Chem 7, and LFTs) regularly throughout the study.

Assessment of Adverse Events

A physician, or designee, will evaluate a subject's AEs based on the NCI CTCAE Version 5.0. A physician will assess whether or not a particular event is likely to be related to the study drug. For more details on event reporting, see Section 7.4.

Monitoring Toxicity

Injection-induced/related serious toxicity will be defined (using CTCAE Version 5.0) as:

- Grade II or higher allergic reactions. Grade II is defined as "Oral intervention indicated". Grade III is defined as "Bronchospasm; hospitalization indicated for clinical sequelae; intravenous intervention indicated".
- Grade II or higher autoimmune reactions/disorders. Grade II is defined as "evidence of autoimmune reaction involving a non-essential organ or function (e.g., hypothyroidism)". Grade III is defined as "autoimmune reactions involving major organ (e.g., colitis, anemia, myocarditis, kidney)".
- Any Grade III or higher event.

Subjects who have any of the above dose-limiting toxicities will be treated and referred for additional care as indicated with systemic steroids, topical steroids, epinephrine or Benadryl. These subjects will no longer receive the vaccine but will continue with assessments per the study calendar, including collection of study labs.

7.3 EFFICACY ASSESSMENTS

Studying the efficacy of PepCan and the schedule in which this protocol specifically outlines the clinical response (i.e., reduced cancer recurrence rate) of subjects is a secondary endpoint. Immune response will be evaluated as the tertiary endpoint, and microbial response will be assessed as the quaternary endpoint. This section includes a list and description of the procedures that will be conducted.

Assessment of Clinical Efficacy

Cancer recurrence rate will be compared for a 2-year period between the PepCan and placebo arms using Fisher's exact test.

Collection of Biological Specimen and Urine Specimen

Urine Specimen

A urine pregnancy test will be administered to subjects of childbearing potential at every visit and the results reviewed by the study team before injection.

Oral Wash Specimen

1. Oral wash specimens will be collected at Screening, Visit 5, Visit 7 and Visit 8.
2. Subjects will be asked to swish 10mL of mouthwash and spit into a cup.

Blood Draw

1. Blood draw will be performed by a trained phlebotomist or other medical professional
2. CBC with differential on Visits Screen, 1, 3, 5, 6, 7, 8, 9
3. Chem 7 on Visits Screen, 1, 3, 5, 6, 7, 8, 9
4. LFTs on Visits Screen, 1, 3, 5, 6, 7, 8, 9
5. 8 rubber green top tubes on Visits 1, 5, 8, 9

Stool Sample

1. Stool samples will be collected with OMNIgene•GUT kit (ref. OMR-200) which includes a toilet accessory sheet, collection tube, and spatula.
2. Subjects will be given appropriate packing and shipping supplies to be sent back to UAMS at Visits Screen, 5, 7, and 8 to the following address:
   Cancer Clinical Trials Office
   4301 West Markham Street, Slot # 724
   Little Rock, AR 72205
3. Instructions will be given to the subject at each visit, but the full instructions for the kit can also be found at: *http://www.dnagenotek.com/US/support/ciOMR200.html*

Future Use of Remaining Samples

Subjects may choose to allow future use of remaining samples in the informed consent form. After all tests are completed, any leftover samples will be saved for future research studies on HPV. The blood, cell, oral, and stool samples will be frozen and will be stored with only the subject identifier. The cell samples may be stored indefinitely until needed for a future research study. Subjects who originally chose to allow future use of remaining samples but change their mind later may notify the PI in writing to the address listed in the informed consent form. The subject identifier will be linked to the subject's name and the study team will collect the sample and destroy it per institutional guidelines.

Laboratory Evaluations

Blood Samples

Complete blood count with differential, Chem 7, and liver function tests will be performed at [INSERT LOCAL LAB] in accordance with their current SOPs.

FACS Analysis

Peripheral blood mononuclear cells (PBMCs) isolated from blood samples drawn at Visits 1, 5, 8, and 9 may be analyzed at UAMS to measure circulating immune cells such as Th1, Th2, regulatory T-cell (Treg), and myeloid-derived suppressor cells (MDSCs). HLA typing may be performed using remaining PBMCs.

ELISPOT

An immune assay such as an ELISPOT assay to assess the presence of HPV-specific T-cells will be performed. After each blood draw, PBMCs will be separated into CD14+ and CD14– populations and cryopreserved. To eliminate inter-assay variability, all 4 blood samples (before vaccination, after 4 vaccinations, 7 vaccinations, and at the end of the study) will be used to establish T-cell lines and to

perform ELISPOT assays. CD3 T-cell lines will be established by stimulating in vitro magnetically selected CD3 cells with autologous mature dendritic cells exposed to HPV-16 E6-vac andE6-GST. ELISPOT assays will be performed at UAMS as previously described [36, 37]. We typically examine 10 regions within the HPV-16 E6 and protein (E6 1–25, E6 16–40, E6 31–55, E6 46–70, E6 61–85, E6 76–100, E6 91–115, E6 106–130, E6 121–145, and E6 136–158). The assay will be performed in triplicate if sufficient cells are available. An HPV-specific T-lymphocyte response will be considered positive if spot-forming units in antigen-containing wells are at least two times higher than corresponding negative-control wells (i.e., a positivity index ≥ 2.0). In order to compare each region before vaccination and after injections (after 4 vaccinations, after 7 vaccinations or at the end of the study), a t test for paired samples will be performed, as described previously [36, 37] for regions with increasing positivity index after vaccinations.

Cytokine/Chemokine Analysis

Plasma samples collected from blood specimens drawn at Visits 1, 5, 8, and 9 may be analyzed at UAMS for cytokine/chemokine panel which may include of IL-1β, IL-1 receptor agonist (IL-1RA), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, eotaxin, basic fibroblast growth factor (FGF), G-CSF, GM-CSF, IFNγ, IFNγ induced protein 10 (IP-10), monocyte chemotactic protein 1 (MCP-1), MIP-1α, MIP-1α, platelet-derived growth factor subunit B (PDGF-BB), regulated on activation, normal T-cell expressed and secreted (RANTES), TNF-α, vascular endothelial growth factor (VEGF), IL-2 receptor α (IL-2Rα), chemokine (C-X-C motif) ligand 1 (CXCL1), hepatocyte growth factor (HGF), IFN-α-2, LIF, chemokine (C-C motif) ligand 7 (CCL7), macrophage migration inhibitory factor (MIF), chemokine (C-X-C motif) ligand 9 (CXCL9), βnerve growth factor (βNGF), stem cell factor (SCF), stem cell growth factor β (SCGF-β), TRAIL, IL-16, and IL-18.

DNA Sequencing of Oral wash and Stool Samples

Extraction of microbial DNA will be performed at UAMS using the MoBio PowerFecal DNA Isolation Kit (QIAGEN, Cat. No. 12830-50, California) or ZymoBioMICS DNA Miniprep Kit (Zymo Research Cat. No. D4300, Irvine, California). Quality of isolated DNA samples may be determined by agarose gel image and OD 260/280 ratio (OD 260/280 = 1.8~2.0). Microbiome diversity analysis may be performed in two methods - Illumina MiSeq/HiSeq and MiniON Flow Cell. For research purposes only, the UAMS bioinformatics researchers will evaluate the quality of data acquisition between two methods of DNA sequencing and provide the microbiome profiling data of DNAs in the fecal samples from subjects with head and neck cancer.

7.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

Definition of Adverse Events (AEs)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

Definition of Serious Adverse Events (SAEs)

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption

of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

To avoid confusion, as the terms "serious" and "severe" are not synonymous, the following clarification is given: The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is *not* the same as "serious," which is based on subject/event *outcome* or *action* usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. *[ICH-E2A(II)(B)]*

Classification of an Adverse Event

Severity of Event

AEs will be graded using the NCI CTCAE Version 5.0. A copy of the NCI CTCAE Version 5.0 can be downloaded from the Cancer Therapy Evaluation Program (CTEP) home page. All appropriate treatment areas have access to a copy of the NCI CTCAE Version 5.0.

Relationship to Study Intervention

All AEs must have their relationship to study intervention assessed by the clinician who examines and evaluates the subject based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** - There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** - There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** - There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** - A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).

- **Not Related** - The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

Expectedness

The local site PI or a local site Sub Investigator will be responsible for determining whether an AE is expected or unexpected. Vaccine-related or possibly vaccine-related AEs, which occurred with ≥5% of injections, in the order of decreasing number of occurrences, were injection-site reaction (immediate and delayed), myalgia, headache, nausea, fatigue, hypokalemia, fever, and flu-like symptoms [36, 37]. None of these AEs was more than grade 2. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study subject presenting for medical care, or upon review by a study monitor.

All AEs occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAEs that are still ongoing at the end of the study period must be followed for up to 30 days to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately to the Sponsor.

Multi-sites will capture adverse event source documentation within their electronic medical record system. Information to be collected may include event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution or to study termination. Additionally, the main site will document AEs in AR-AERS and the external sites will document AEs on a provided AE Log to ensure that all information is captured uniformly across multi-sites. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate electronic Case Report Form (eCRF) in OpenClinica (OC).

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the study subject's condition deteriorates or exacerbates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Study staff responsible for reporting AEs will be listed on the Delegation Log and will record all reportable events with start dates occurring any time after informed consent has been obtained until the end of study participation. At each study visit, the investigator, or a designee, will inquire about the occurrence of

AEs/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

A summary of AEs will be included in the annual IRB status report and the IND report to the FDA.

Serious Adverse Event Reporting

A serious, unexpected (previously not expected in nature, severity, or degree of incidence), and drug-related AE must be reported to:

- ORRA and UAMS PI within 24 hours of local site PI being notified.
- The FDA will be notified using the MedWatch Form FDA 3500A within 10 days of local site PI being notified. The local site PI will report to ORRA and UAMS PI; ORRA will report to FDA.
- The UAMS IRB will be notified of events requiring expedited reporting within 10 days of local site PI being notified (see below if SAE is death).
- A drug-related death or event that is considered life-threatening occurring while a subject is on the study must be reported to the UAMS IRB immediately and to the FDA within 7 days of the investigator learning of the event.
- THE CAUSE OF DEATH AND THE INVESTIGATOR'S DISCUSSION REGARDING WHETHER OR NOT THE DEATH WAS DRUG-RELATED SHOULD BE DESCRIBED IN A WRITTEN REPORT.

7.5 ELECTRONIC DATA CAPTURE

OpenClinica

OpenClinica (OC) is an open source clinical trial software platform and will be the Electronic Data Capture (EDC) for this study's clinical data management. OC will be the main platform for external sites to report data to the main site. External sites will register subjects to the study through OC. Multi-sites will capture source documentation within their electronic medical record system and report all necessary safety and clinical data to the main site by completing the appropriate eCRFs.

7.6 QUESTIONNAIRES

Demographics and Contact Information Form

The Demographics and Contact Information Form serves to collect information regarding patient name, date of birth, race, ethnicity, phone number, and email address. This form will be completed at the screening visit. The purpose of obtaining this information is to obtain updated patient demographic and contact information for the study.

Screening Visit Questionnaire

The Screening Visit Questionnaire serves to collect information regarding HPV vaccination status, recruitment, employment, education, dependents, sexual life, tobacco usage, and alcohol usage. The purpose of obtaining this information is to characterize the subject population and to identify variables that may be associated with vaccine response.

Oral Wash Antibiotic Usage Questionnaire

The Oral Wash Antibiotic Usage Questionnaire serves to collect information regarding patient antibiotic usage within the last 30 days, mouthwash usage, and details for oral wash collection sample. The purpose of obtaining this information is to aid in the interpretation of microbiome data as antibiotics are known to drastically alter them.

Stool Sample Antibiotic Usage Questionnaire

The Stool Sample Antibiotic Usage Questionnaire serves to collect information regarding patient antibiotic usage within the last 30 days. The purpose of obtaining this information is to aid in the interpretation of microbiome data as antibiotics are known to drastically alter them.

Visit 9 Questionnaire

The Visit 9 Questionnaire serves to collect information regarding patient experience such at the end of study treatment. The purpose of obtaining this information is to improve retention.

Recurrence Questionnaire

The Recurrence Questionnaire serves to collect information regarding patient experience such at the end of study treatment. The purpose of obtaining this information is to improve retention.

Early Termination Questionnaire

The Early Termination Questionnaire serves to collect information regarding reasons why patients are withdrawing their participation on study prior to completion of study treatment. The purpose of obtaining this information is to improve retention.

8. STATISTICAL CONSIDERATIONS

8.1 DETERMINATION OF SAMPLE SIZE

The recurrence rate of head and neck cancer is 80% over 2 years (50 to 60% local recurrence and 20 to 30% distant recurrence [41]. In the Phase I trial of PepCan described above the overall histological regression rate of HSILs was 45% [36]. A reduced recurrence rate by the magnitude of 45% would reach 44%. Vaccinating 72 subjects in the PepCan arm and 24 subjects in the placebo arm would have 90% power at alpha of 5%, 2 tailed. Accounting for about 5% drop out rate, 75 subjects would be needed in the PepCan arm, and 25 subjects in the placebo arm.

8.2 STATISTICAL ANALYSES

Subjects who received at least one dose of the vaccine will be included in safety assessments. Safety analyses will include summaries of the incidence of AEs using the NCI CTCAE Version 5.0 that occur during the study period regardless of causality. The AEs will be mostly descriptive initially. However, after unblinding at the completion of the study, rates of common side effects (occurring with 5% or more injections) will be compared between the PepCan and the placebo arms. Efficacy will be assessed by comparing cancer recurrence rate between the 2 arms. The expected number of head and neck cancer patients who achieve remission is 100 patients per year. For immunological assessments, the presence of new anti-HPV-16 E6 T-cell activity will be measured using CD3 ELISPOT assay, and its statistical significance will be assessed using a paired *t*-test. For analyses of immune cells such as Th1, Th2, Treg, and MDSCs, percentages before and after vaccination will also be assed using a two-tailed paired *t*-test. The frequencies between subjects who experience recurrence and those that do not in the PepCan arm will be compared using Wilcoxon rank-sum test. The diversity of gut microbiome will be assessed prior to

injection, and will be compared to that after injection using alpha diversity, beta diversity, and compositions at the phylum or family levels. These parameters will also be compared between subjects with and without recurrence with in the PepCan arm.

9. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

9.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

Institutional Review Board and Multi-sites

UAMS IRB will serve as the IRB of record for all multi-sites. (should the study expand to additional sites) UAMS will serve as the main site. The main site, specifically the UAMS CTO Regulatory Unit, will be responsible for submitting documents to the UAMS IRB on behalf of the multi-sites and communicating to the multi-sites information from the UAMS IRB.

Multi-sites are expected to vaccinate 27 participants per site. When this goal is reached, each site can request to increase their goal as long as the overall enrollment has not been met. Multi-sites will use OpenClinica to enter safety and clinical data. All research laboratory analysis will be performed at or by UAMS, and the results will be shared with multi-sites as needed.

Informed Consent Process

Consent and Other Informational Documents Provided to Subjects

Consent forms describing in detail the study intervention, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study intervention. Any revisions of the consent form will be provided to the subjects as deemed necessary by the IRB or Sponsor before any subsequent trial interventions take place, and all consent form documents will be kept in the subject's binder.

Consent Procedures and Documentation

Consent forms will be Institutional Review Board (IRB)-approved and the potential subject will be asked to read and review the document. The investigator will explain the research study to the potential subject and answer any questions that may arise. A verbal explanation will be provided in terms suited to the potential subject's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research subjects. Potential subjects will have the opportunity to carefully review the written consent form and ask questions prior to signing. The potential subject should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate.

If the potential subject agrees to participate, he/she will sign the informed consent document prior to any procedures being done specifically for the study. Potential subjects must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to subjects for their records. The informed consent process will be conducted and documented in the source document (including the date). The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

9.2 STUDY DISCONTINUATION AND CLOSURE

Subjects are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue a subject's participation from vaccination or from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance
- Any subject experiencing a vaccine-related toxicity
- Disease progression, and/or unrelated health condition which requires discontinuation of the study intervention
- Any reason that the investigator feels it is not in the subject's best interest to continue

The sponsor may decide to stop the study at any point, for any reason.

INDIVIDUAL SUBJECT STOPPING RULES

Subjects who experience injection-induced/related serious dose limiting toxicities should be removed from the study treatment (vaccine) but will continue with assessments per the study calendar, including collection of study labs. Injection-induced serious toxicity will be defined (using CTCAE Version 5.0) as:

- Grade II or higher allergic reactions. Grade II is defined as "Oral intervention indicated". Grade III is defined as "Bronchospasm; hospitalization indicated for clinical sequelae; intravenous intervention indicated".
- Grade II or higher autoimmune reactions/disorders. Grade II is defined as "evidence of autoimmune reaction involving a non-essential organ or function (e.g., hypothyroidism)". Grade III is defined as "autoimmune reactions involving major organ (e.g., colitis, anemia, myocarditis, kidney)".
- Any Grade III or higher event.

If a subject becomes pregnant during her participation in the study, a medical monitor will determine whether or not she should be removed from the study. Medical records will be requested to determine the health of the mother and child.

For all other circumstances, the local site PI may determine whether or not a subject needs to be removed from the study. In either case written notification, documenting the reason for study suspension or termination, will be provided to the study subject, Investigator, ORRA, and local regulatory authorities. If a subject withdraws or is withdrawn, they will be asked to complete the Early Termination Questionnaire. Withdrawn subjects will not be replaced.

STUDY STOPPING RULES

If at any point in the study, ≥ 10% of subjects experience injection-related serious toxicity, the study enrollment and injections will be suspended. These activities can only re-start after notifying the applicable regulatory agencies and with a permission to resume from the Medical Monitor and Sponsor.

The study enrollment and injections will be suspended if any subject experiences vaccine-related Grade IV or higher AE. These activities can re-start only after notifying the applicable regulatory authorities and with a permission to resume from the Medical Monitor and Sponsor.

If the study is prematurely terminated or suspended, the UAMS PI will promptly inform study subjects, the IRB, and Sponsor and will provide the reason(s) for the termination or suspension. Study subjects will be contacted, as applicable, and be informed of changes to study visit schedule. If only suspended, the study may resume once all concerns have been addressed, and satisfy the Sponsor, IRB and/or the FDA.

Emergency Unblinding

If a medical emergency necessitating the identity of the injection administered occurs, the local site PI or a local Sub-Investigator will notify the sponsor and UAMS PI. The UAMS PI will be available 24 hours, 7 days a week, and if the medical emergency occurs after hours the UAMS PI should be notified via the after-hours number listed on the protocol title page. After permission is given to unblind by the UAMS PI, local site pharmacy will identify the injection to the requesting investigator and Study Coordinator, and note any incidence in the Randomization book. UAMS PI will notify the medical monitor if necessary for further review.

Lost to Follow-Up

A subject will be considered lost to follow-up if he or she fails to return for two (2) consecutive scheduled visits and is unable to be contacted by the study site staff.

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

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

Travel Stipends

Travel stipends will be available based on institutional guidelines

Confidentiality and Privacy

Subject confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to subjects. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All study related documents will be kept in accordance with local institutional practices.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The study subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

KEY ROLES AND STUDY GOVERNANCE

UAMS Principal Investigator (PI)	**Medical Monitor**
Omar Atiq, MD	Rangaswamy Govindarajan, MD
University of Arkansas for Medical Sciences Winthrop P. Rockefeller Cancer Institute 7th Floor 4301 W. Markham Little Rock, AR 72205	University of Arkansas for Medical Sciences Winthrop P. Rockefeller Cancer Institute 7th Floor 4301 W. Markham Little Rock, AR 72205

MONITORING

The UAMS and local PIs will have the overall responsibility for assuring safety and gathering the data for the study with assistance from the sub-investigators, and research staff, under the guidance of the Institutional Review Board (IRB) and the study Sponsor.

Clinical site monitoring for the main site, UAMS, is conducted by ORRA to ensure that the rights and well-being of human subjects are protected; that the trial data are accurate, complete, and verifiable from source documents; and that the trial is conducted in compliance with the currently approved protocol/amendment(s), ICH GCP, and applicable regulatory requirements.

Clinical site monitoring for the external multi-sites is conducted by Marshall and Associates to ensure that the rights and well-being of human subjects are protected; that the trial data are accurate, complete, and verifiable from source documents; and that the trial is conducted in compliance with the currently approved protocol/amendment(s), ICH GCP, and applicable regulatory requirements. Monitoring specialists from Marshall and Associates will conduct periodic on-site, comprehensive monitoring as determined by a protocol-specific monitoring plan provided by the Sponsor,

The designated medical monitor will serve as the medical monitor for all sites. Safety and clinical data reported by the external multi-sites to the main site will be reviewed by the UAMS PI. The UAMS PI will notify the medical monitor of any AEs that will require their medical expertise, oversight, and consultation to ensure the safety and integrity of the subjects throughout the trial from the initial design of the study to the final close-out. Such AEs would include, but not limited to, any dose-limiting toxicities and unintended pregnancies. The dose-limiting toxicities are defined as vaccine-related allergic and autoimmune AEs greater than grade 1 and any other AEs greater than grade 2.

Quality Assurance and Quality Control

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, recorded, and reported in compliance with the protocol and all applicable regulatory requirements.

Data Handling and Record Keeping

Data Collection and Management Responsibilities

The investigator shall maintain a list of appropriately qualified persons to whom he has delegated trial duties. All persons authorized to make entries and/or corrections on case report forms will be included on the Delegation of Authority form.

The investigator and study staff are responsible for maintaining a comprehensive and centralized filing system of all study-related documentation.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each subject enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be collected.

Protocol Deviations and Violations

A deviation is a study event that is not covered under the existing protocol and represents a failure to comply with the protocol.

A violation is an event clearly occurring outside of the approved research activity, which also represents a failure to comply with the protocol, e.g., enrollment of a subject that fails to meet inclusion or exclusion criteria. A protocol violation refers to more serious non-compliance, which more often leads to exclusion of subjects from eligibility analysis or their discontinuation from the study.

If the protocol deviation/protocol violation does not represent a significant alteration in the approved protocol and/or affect the safety or welfare of the subject, it will be reported to the UAMS IRB at the time of Continuing Review.

If the protocol deviation or protocol violation represents a significant alteration in the approved protocol and/or if it affects the safety or welfare of the subject, it must be reported to the UAMS IRB immediately.

Multi-sites may capture protocol deviations and violations source documentation within their electronic record system. Additionally, the main site will document protocol deviations and violations in RPRS and the external sites will document protocol deviations and violations on a provided Protocol Deviations and Violations Log to ensure that all information is captured uniformly across multi-sites.

Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

- National Institutes of Health (NIH) Public Access Policy
- NIH Data Sharing Policy
- Policy on the Dissemination of NIH-Funded Clinical Trial Information
- Clinical Trials Registration and Results Information Submission rule

Results will be submitted for publication in peer-reviewed journals and/or presented at professional society meetings at national and international levels.

Conflict of Interest Policy

The study leadership in conjunction with the UAMS IRB has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest. This research study is designed to test a product invented by Dr. Nakagawa. UAMS and Dr. Nakagawa are entitled to a share of royalties received from the sale of this product. The financial value of this interest might be affected by the results of this study. This means that UAMS and Dr. Nakagawa could gain or lose money depending on the results of this study.

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11. APPENDICES

11.1 SCHEDULE OF STUDY ASSESSMENTS

		Scheduled after stool sample received	Scheduled 3 weeks after previous visit (±7 days)			Scheduled 3 months after previous visit (±14 days)			Scheduled 6 months after previous visit (±30 days)	
	Screening Visit	**Visit 1[a]**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**
Informed Consent[b]	X									
Inclusion/Exclusion Review	X									
Demographics and Contact Information Form	X									
Medical History[c]	X									
Screening Visit Questionnaire	X									
Physical Examination[d]	X					X	X	X	X	X
CBC w/ diff, Chem 7 and LFT[e]	X	X		X		X	X	X	X	X
Blood for Immunological Assessment[f]		X				X			X	X
Urine Pregnancy Test	X	X[g]	X	X	X	X	X	X	X	X
In-Clinic Vital Signs[h]	X								X	X
Adverse Events		X	X	X	X	X	X	X	X	X
Concomitant Medications	X[i]	X	X	X	X	X	X	X	X	X
Pre-Injection Vital Signs[j]		X	X	X	X	X	X	X		
Vaccination		X	X	X	X	X	X	X		
Post-injection Vital Signs[j]		X	X	X	X	X	X	X		
Analgesics offered		X	X	X	X	X	X	X		

[a] Visit 1 must be scheduled within 90 days of the screening visit.

[b] Subjects will sign a revised Informed Consent Form, when applicable.

[c] Medical History will include a specific review of past cancer diagnosis, treatment, remission. Note specific tests, including performance evaluation, ENT evaluation, and radiography, to support No Evidence of Disease (NED) status. Note p16 and/or HPV status of tumor, if available.

[d] Physical examination of the head and neck performed during screening visit or within 30 days prior to the subject signing consent and as standard of care at visits 5-9.

[e] CBC to include white count, hemoglobin, hematocrit, and platelet count; Chem 7 to include BUN, calcium, CO2, chloride, creatinine, sodium and potassium; LFT to include alk phos, total bili, LD, AST and ALT. Standard of care clinical labs may be used for the screening visit labs if performed within 30 days prior to the subject signing consent.

[f] Eight rubber green top (sodium heparin) tubes will be drawn for ELISPOT assay and FACS analysis at visits 1, 5, 8, 9.

[g] To be performed prior to vaccine administration for women of childbearing potential.

[h] In-clinic vital signs will include blood pressure, heart rate, respiratory rate and body temperature. If screening vital signs are obtained within 30 days prior to the subject signing consent then those results can be used to confirm eligibility. If screening vital signs were not collected within 30 days prior to the subject signing consent, they must should be obtained at screening visit. For screening vital signs outside of the acceptable ranges, they can be repeated within 30 days of signing the consent OR.

[i] Screening concomitant medications can be collected if it is done within 30 days prior to the subject signing consent

[j] Pre- and post-injection vital signs will include blood pressure, heart rate, respiratory rate and body temperature

Visit 9 Questionnaire										X
Oral Wash Sample Collection[k]	X					X		X	X	
Dispense Stool Sample Kit[l]	X					X		X	X	

[k] Subjects will swish 10 mL of mouthwash, spit into a cup, and complete an oral wash antibiotic usage questionnaire.

[l] The stool collection kit, including a stool sample antibiotic usage questionnaire, will be provided with instructions for collection and shipping.

12. PROTOCOL AMENDMENT HISTORY

Version	Date	Brief Description of Change
2	04/08/2019	Added NCT number; clarified definition of serious toxicity; clarified monitoring; corrected schedule of study assessments footnotes j and k.
3	06/03/2019	Changed study design to randomized double-blind placebo control; added placebo description; added oral wash, antibiotic usage questionnaire and stool sample collection at Visit 7; increased time required after brushing to 2 hours; clarified that control subjects will be in the placebo arm; added 5.5 Recurrence Section and "Recurrence Questionnaire"; added 5.6 Emergency Unblinding section; clarified Individual Subject and Study Stopping Rules and under section 9.2 Study Discontinuation and Closure.
4	08/20/2019	Clarified eligibility criteria for consistency; clarified Oral Wash Antibiotic Usage Questionnaires and Stool Sample Antibiotic Usage Questionnaires; changed reference to Form FDA 3500A and clarified who reports to whom; corrected green top tubes to be used in study calendar footnotes
5	11/18/2019	Removed "Current use of beta-blocker medication" exclusion criteria
6	01/23/2020	Added one blood pressure recheck during screening visit; added possible re-enrollment as new subjects for BP screen failures upon documentation of controlled BP within the acceptable range (≤160/95 mm Hg); added 30-day window prior to screening visit blood draw to use standard of care clinical labs.
7	02/18/2020	Changed inclusion criteria for completing curative therapy to within 120 days, as 90 days was insufficient for subjects to recover from the effects of previous treatments. Added 90 window between screening and visit 1.
8	03/17/2020	Removed of anion gap & eGFR. Added of 30-day allowance prior to screening for the physical exam. Added statement to allow study staff to contact potential subject prior to screening visit. Corrected footnotes on schedule of study assessments.
9	03/04/2021	Revised Inclusion Criteria #3. Added sub-investigators. Added Travel Stipends section (updated from consent document).
10	06/17/2021	Revised Section 7.2 to clarify measures taken for patients who have dose limiting toxicities.
11	12/09/2021	Removed of 20 mL research blood draws from visits 3, 6, and 7.
12	08/03/2022	Master protocol, ICF, and CRF templates for multi-site use.
13	08/22/2022	Addressed IRB contingencies for master protocol, ICF, and CRF templates for multi-site use.
14	09/15/2022	Addressed IRB contingencies for master protocol template for multi-site use.
15	09/28/2022	Added emergency unblinding from the UAMS PI and Research Pharmacy for local investigator.
16	10/05/2022	Clarification in emergency unblinding section to state that the UAMS PI will give permission to unblind and will be available 24/7.
17	02/01/2023	Updated screening vital sign ranges for inclusion and exclusion criteria. Specified that screening vitals obtained with 30 days of the subject singing the consent can be used for eligibility, and repeat visit for out of range vital signs is acceptable. Subject can be enrolled if the Medical Monitor determines it is safe. Specified that medical history and concomitant medications can be collected if it is done with 30 days of the subject singing the consent. Removed information regarding BP for screen failures in Section 5.3 and Section 7.1.
18	06/21/2023	Updated the Section 7.2 Monitoring Toxicity sub-section to include additional Grade II (oral intervention indicated) and Grade III (bronchospasm) events that are related serious toxicities. Added a new sub-section called Future Use of Remaining Samples to Section 7.3 to provide information about future use of samples and to match the informed consent form. Updated the Schedule of Study Assessments to correct footnotes.
19	01/05/2024	Updated study statistician to Milan Bimali.