A Phase II Clinical Trial of PepCan Randomized and Double-Blinded to Two Therapy Arms for Treating Cervical High-Grade Squamous Intraepithelial Lesions

UAMS IRB 202790

NCT Number: NCT02481414

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SPONSOR:
University of Arkansas for Medical Sciences

FUNDING SOURCE:
National Institutes of Health (National Cancer Institute)

LOCATION OF STUDY:
University of Arkansas for Medical Sciences
Obstetrics and Gynecology Clinics
UAMS Winthrop P. Rockefeller Cancer Institute (WPRCI)

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GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
ALT	alanine transaminase
ANGELS	UAMS Antenatal Guidelines, Education and Learning System
AST	aspartate transaminase
BMI	body mass index
Bx	biopsy
cGMP	current good manufacturing practice
CBC	complete blood count
CIN	cervical intraepithelial neoplasia (Grade 1, 2, or 3)
CLARA	CLinicAl Research Administrator
COD	clinically optimal dose
CRF	case reporting form
CTCAE	Common Terminology Criteria for Adverse Events
CTL	cytotoxic T-lymphocyte
DNA	deoxyribonucleic acid
DC	dendritic cell
DLT	dose limiting toxicity
ECC	endocervical curettage
EDTA	ethylenediaminetetraacetic acid
ELISPOT	enzyme-linked immunospot
FACS	fluorescence-activated cell sorting
FDA	United States Food and Drug Administration
GCP	good clinical practice
GGT	gamma-glutamyl transpeptidase
GLP	good laboratory practice
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSIL	high-grade squamous intraepithelial lesion
HPV	human papillomavirus
HPV 16	human papillomavirus type 16
IFN-γ	interferon-γ
IND	investigational new drug
IOD	immunologically optimal dose

IRB	institutional review board
LEEP	loop electrical excision procedure
LCs	Langerhans cells
LSIL	low-grade squamous intraepithelial lesion
MDSC	myeloid-derived suppressor cells
MTD	maximum tolerated dose
NIH	National Institutes of Health
NCI	National Cancer Institute
NS	not significant
NSAIDS	non-steroidal anti-inflammatory drugs
OBGYN	Obstetrics and Gynecology
OR	Operating Room
Pap	Papanicolaou
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PI	principal investigator
PPD	purified protein derivative
PRMC	Protocol Review and Monitoring Committee
SIL	squamous intraepithelial lesion
Treg	regulatory T-cells
UAMS	University of Arkansas for Medical Sciences
UPT	urine pregnancy test
WPRCI	Winthrop P. Rockefeller Cancer Institute

1 BACKGROUND

1.1 STUDY SYNOPSIS

Title	A Phase II Clinical Trial of PepCan Randomized and Double-Blinded to Two Therapy Arms for Treating Cervical High-Grade Squamous Intraepithelial Lesions
IRB Number	202790
Methodology	This is a Phase II study to evaluate the efficacy and safety of an HPV therapeutic vaccine called PepCan (HPV 16 E6 peptides combined with *Candida* skin testing reagent called Candin®) in adult females over a 12-month time period. As the results from the Phase I trial demonstrated some efficacy against non-16 HPV types, Candin® alone will also be tested. Therefore, there will be two treatment arms: (1) PepCan and (2) Candin®. Subjects found to be eligible for vaccination will be randomized in a double-blinded fashion at a 1:1 ratio. Each subject will be receiving injections four times with three weeks between injections. Clinical and virological responses will be assessed at 6- and 12-months. Safety will be assessed from the time of enrollment to 12-Month Visit. Immunological assessments will be made at 4 time points (prevaccination, after 2 injections, 6 months after 4 injections and 12 months after 4 vaccinations).
Study Duration	66 months
Study Center	University of Arkansas for Medical Sciences
Objectives	Primary Objective - To evaluate the efficacy of PepCan in humans Secondary Objectives - To evaluate the safety of PepCan in humans Tertiary Objective - To evaluate immunological response and viral clearance Other Outcome Measures - To identify factors predicting clinical and virological responses to PepCan; to evaluate vaccine effect; to examine mechanisms of cross-protection
Outcome Measures	Primary Outcome Measure: Clinical response as assessed by histological regression of HSIL at 12 months Secondary Outcome Measure: Safety Tertiary Outcome Measures: Immunological response and viral clearance Other Outcome Measures: Predicting vaccine response using various factors such as age, oral contraceptive use, smoking history, circulating immune cells, HLA types, HPV types, bacterial taxa, and cytokine/chemokine profiling; evaluating vaccine effect; determining cross-protection and examining epitope-spreading and cross-reactivity as possible mechanisms
Number of Subjects	125 adult women will be screened; up to 80 women will participate in the vaccination phase and will be randomized to PepCan and Candin® arms
Diagnosis and Main Inclusion Criteria	Recent Pap smear result consistent with HSIL or "cannot rule out HSIL" or recent untreated colposcopy guided biopsy-confirmed HSIL
Eligibility for Vaccination	Recent untreated colposcopy guided biopsy-confirmed HSIL
Study Product(s), Dose, Route, Regimen	Test Article: Vaccine consisting of four HPV 16 E6 peptides in combination with Candin® (PepCan) or Candin® alone Route of Administration: Intradermal injection Peptide Dose Level: 50 µg/peptide/injection based on the clinically optimal dose from the Phase I study Candin® Dose Level: 300 µl/injection for PepCan and Candin® groups Dosing Regimen: 4 injections; three weeks between each injection Injection site: Limbs
Statistical Methodology	Clinical response data (PepCan or Candin®) will be compared to a historical placebo control group from a similarly designed clinical trial using appropriate statistical tests; measures of safety will be evaluated using descriptive statistics.

1.2 SUMMARY

Cervical cancer is the fourth most common malignancy in women worldwide, and 528,000 new cases are diagnosed annually and approximately 266,000 deaths occur annually from the disease [1]. The link between human papillomavirus (HPV) infection and the development of cervical cancer is well known [2]. Among more than 100 different types of HPV, at least 15 are strongly associated with invasive squamous cell cancer of the cervix [3], with human papillomavirus type 16 (HPV 16) being the most common of these [4-6]. HPV infection is also associated with the precursor lesion of cervical cancer, squamous intraepithelial lesion (SIL) [4-9]. Most low-grade squamous intraepithelial lesions (LSILs) regress spontaneously [10, 11], though some do progress to high-grade squamous intraepithelial lesions (HSILs). These high-grade lesions, particularly cervical intraepithelial neoplasia (CIN) 3, are associated with high rates of progression to invasive cervical cancer [12, 13].

Although prophylactic vaccines that would prevent HPV infection and subsequent development of cervical cancer are available, they are not effective in individuals who already have acquired HPV. Therefore, a therapeutic vaccine, which can treat those who are already infected, including women diagnosed with HSIL, is needed but none is clinically available. Such a vaccine would be expected to benefit young women (narrowly defined as ≤24 years old and broadly defined as any women who still plans to become pregnant[14]) since a recently recognized and unintended side effect of surgical treatments for HSIL such as LEEP is increased incidence of preterm delivery from 4.4% to 8.9%[14, 15]. Therefore, the new treatment guidelines published in 2013 recommends one to two years of close observation in young women with cervical intraepithelial neoplasia (CIN) 2. Furthermore, an HPV therapeutic vaccine, which only requires injections, can benefit women from developing regions where surgical expertise may not be available to perform excisional procedures.

Our group has developed a vaccine consisting of four clinical good manufacturing practice (cGMP) grade synthetic peptides covering the HPV type 16 E6 protein and *Candida* skin test reagent as a novel vaccine adjuvant (named PepCan). The dose-escalation portion of the Phase I clinical trial of the therapeutic vaccine treating women with biopsy-confirmed HSILs has recently been completed (the final dose phase using the clinically optimal dose of 50 μg per peptide is still ongoing). No dose-limiting toxicities (DLTs) [vaccine-related allergic and autoimmune adverse events (AEs) > Grade 1 and any other AEs > 2 Grade 2] have been observed. The highest % of histological regression was observed in the lowest dose (50 μg per peptide) group (5 of 6 or 83%) with the overall (50, 100, 250, and 500 μg per peptide doses were tested) response rate of 52% (12 of 23). Both these rates are above that of a historical placebo group of 22% [16]. Both subjects with HPV 16 and non-16 HPV-positive HSILs responded to PepCan. While we are assessing histological response 3 months after vaccination, the full effect takes 1 year [17-19] to manifest as demonstrated by increase in histological regression from 25% to 47% of vulvar intraepithelial lesions treated by a similar vaccine [18].

This phase II clinical trial aims to assess the full clinical efficacy of PepCan by assessing response at 1 year, to identify factors, which can predict favorable vaccine response, examine vaccine effects, and to define against which non-16 HPV types this vaccine is effective. Candin® alone will also be examined. Approximately 125 subjects will be screened and 80 subjects will be vaccinated over 3.5 to 4.5 years. If needed additional year can be added.

1.3 SCIENTIFIC RATIONALE

1.3.1 Rationale for Using HPV Peptides as the Antigen

1.3.1.1 Importance of the T-Cell Responses to HPV 16 E6 Protein in Viral Clearance

The National Cancer Institute (NCI)-supported study conducted by Anna-Barbara Moscicki, MD, was one of the first to describe the relationship between viral persistence and development of SILs [20-22]. Women were recruited at the San Francisco State University Medical Clinic and the Hayward Planned Parenthood Clinic. A total of 654 women were actively followed via clinic visits every 4 months. At these visits, a sexual-history interview, Pap smear, colposcopy, and HPV-deoxyribonucleoic acid (DNA) testing on cervical lavage specimens were performed. Mayumi Nakagawa, MD, PhD [principal investigator (PI)], first studied T-cell immunity against HPV in this cohort.

HPV 16 E6- and E7-specific cytotoxic T lymphocytes (CTLs) were demonstrated in subjects who had evidence of HPV 16 infections but had not developed SILs [23-25]. In a small cross-sectional study, the percentage of subjects who demonstrated HPV 16 E6- and/or E7-specific CTLs was higher in the group of women with HPV 16 infections without SILs than in the group of women with HPV 16 infections who developed SILs [23].

In women with polymerase chain reaction (PCR)-detected cervical HPV 16 infections (from the same cohort described above), we examined the association between HPV 16 E6- and E7-specific CTLs and HPV 16 persistence, using a longitudinal study design involving multiple CTL assays [25]. Women with HPV 16 infections (n=51) were enrolled, along with women who were HPV 16 negative as controls (n=3). Twenty-two of 40 (55%) women whose HPV 16 infections had cleared each had at least one E6 CTL response, while none of the nine women who had HPV 16 persistence had such a response (p=.003). This difference was not demonstrated for E7; 25 of 40 (63%) women whose HPV 16 infections cleared had E7 CTL responses, and five of nine (56%) women with persistent infections had responses (p=.720). Therefore, **the CTL response to E6 appears to be important in clearing HPV 16 infection**. We also examined whether these T-cells were CD4- and/or CD8-positive using antibody blocking and T-cell subset separation experiments, and we demonstrated that both CD4- and CD8-positive T-lymphocytes demonstrated anti-HPV activities [24].

In a subsequent study (same cohort) with the same design, we found similar results using *ex vivo* interferon (IFN)-γ enzyme-linked immunospot (ELISPOT) assays instead of CTL assays [26]. Fourteen of 24 (58%) women whose HPV 16 infections cleared each had an E6 response at least once, while none of 10 (0%) women who had HPV 16 persistence had a response (p=.002). For E7, 8 of 24 (33%) women whose HPV 16 infections cleared each had an E7 CTL response, and none of 10 (0%) women with persistence had such a response (p=.04). These results confirmed the importance of T-cell responses to E6 and indicated that E7 may also play a role.

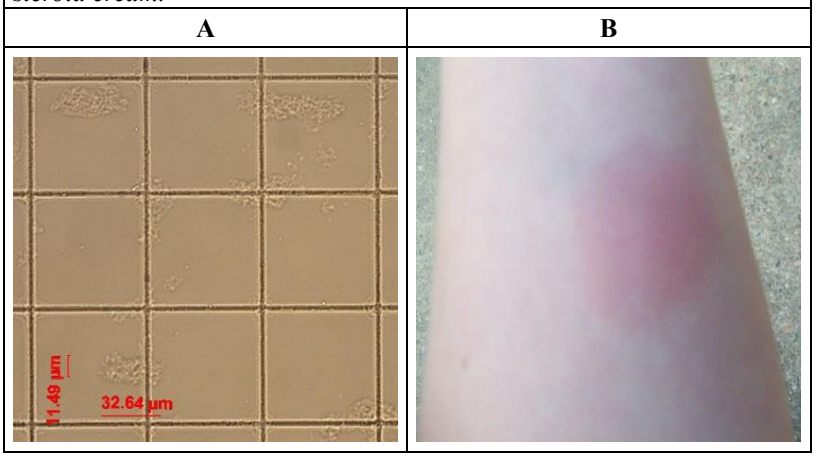
1.3.1.2 Importance of the CD8 and CD4 T-Cell Responses to HPV 16 E6 in Regression of Cervical Lesions

Eighty-five subjects with recent histories of untreated abnormal Pap smears were recruited from the University of Arkansas for Medical Sciences (UAMS) Obstetrics and Gynecology Clinics between 1/11/07 and 7/15/08. HPV-DNA tests using the Linear Array HPV Genotyping Test (Roche Diagnostics, Indianapolis, IN) and ELISPOT assays using the HPV 16 antigens were performed on cervical cytology specimens (ThinPrep, Cytyc Corporation, Marlborough, MA) [27, 28]. From whole-blood–derived peripheral blood mononuclear cells (PBMCs), CD8 T-cell lines were established by two rounds of in vitro stimulation of magnetically selected CD8 T-cells with autologous mature DCs infected with recombinant vaccinia viruses expressing E6 or E7 (E6-vac

or E7-vac), and the pattern of immunodominance was examined with ELISPOT assays using 15-mer peptides that overlap by 10 amino acids [28]. Evidence of potential antigenic epitopes was defined by spot-forming units greater than twice the amount for the no-peptide control. The subjects with subsequent normal histological diagnoses were considered to be regressors (n=28) while those with histological diagnoses of CIN 1, 2, or 3 were considered to be persistors (n=37). The indeterminate group included subjects (n=20) for whom diagnoses were equivocal (such as atypical squamous cells of undetermined significance but not excluding HSIL) or for whom insufficient samples were submitted.

CD8 T-cell responses to the HPV 16 E6 antigen were significantly higher for the regressor group compared to the persistor group (54% vs. 24%, p=.04), but this was not observed for the E7 antigen (11% for the regressor group, 12% for the persistor group; p=1.00). The results were the same when the analyses included only subjects who were positive for high-risk HPV (n=48, p=0.01 for E6 and p=0.64 for E7). These results suggest not only that CD8 T-cell responses to HPV 16 E6 are significantly associated with regression of cervical lesions, but also that such protective responses may be cross-reactive among high-risk HPV types. All regions were shown to be immunogenic, but immune responses were most frequently detected against E6 91–115 (n=11), E7 46–70 (n=10), and E6 46–70 (n=8). Similar results were obtained when CD4 T-cell responses were studied in 84 additional subjects from the same clinic [29]. Significantly higher responses were seen in the regressor group for the E6 antigen compared to the persistor group (45% vs. 20%, p=0.02). Again no such difference was seen for E7 (15% vs. 6%, p=0.25). Therefore, HPV 16 E6 protein would be an ideal antigen for a therapeutic HPV vaccine.

Fig. 1 *Vaccine properties.* ***A.*** *Microparticles formed when the vaccine peptides with and without adjuvant were placed in neutral pH in vitro.* ***B.*** *A delayed injection site reaction appearing 5 days after the 1st injection at the 50-μg dose. Such reactions are treated with ice packs and topical steroid cream.*



In vitro investigation has unexpectedly revealed that the four cGMP peptides covering the HPV 16 E6 protein has maturation effects on Langerhans cells (LCs) as measured by up-regulated CD40 ($p=0.00007$) and CD80 ($p<0.00001$) levels [30]. These maturation effects are likely to be due to the formation of microparticles (Fig. 1) by peptides (which are soluble in acidic pH of the formulation) at a neutral pH. As insoluble microparticles are likely to be phagocytosed by LCs resulting in their activation and antigen presentation, the immediate and delayed injection site reactions observed during the Phase I clinical trial (Fig. 1) may be due to these microparticles.

1.3.2 Rationale for Using Candin® as a Vaccine Adjuvant

1.3.2.1 Search for a New and Effective Vaccine Adjuvant

Four current good manufacturing practice (cGMP)-grade synthetic peptides, covering the HPV 16 E6 protein, are incorporated into PepCan since it has been shown to contain CD8 and CD4 epitopes associated with SIL regression. The advantages of using synthetic peptides are (1) ease of producing cGMP-grade material, (2) general safety profiles in previous clinical trials [31-38], and (3) much lower concern for oncogenicity of the E6 protein, which have mutagenic properties as whole proteins.

The most widely used adjuvant in approved human vaccines is an alum-based adjuvant that has been shown to elicit a predominantly Th2 immune response [39]. Therefore, the alum-based adjuvant would be useful in a vaccine designed to boost antibody responses, but not for a vaccine designed to stimulate cellular immune responses. Since successful clearance of HPV infection is believed to be induced by cell-mediated immunity [17, 18], an adjuvant that would promote such immunity is necessary.

Traditionally, recall antigens, which typically include a panel of Candida, mumps, and Trichophyton, were used as a control to indicate an intact cellular immunity when patients were being tested for Tuberculosis by placement of PPD intradermally. T-cell mediated inflammation would become evident in 24 to 48 hours [40]. A number of studies, mostly from UAMS, have demonstrated that recall antigen injections can also be used to treat common warts [41-46]. Furthermore, several studies have shown that the treatment of warts with recall antigens to be effective for not only injected warts but also distant untreated warts [41-45, 47]. This suggested that T-cells may have a role in wart regression. In a recently completed Phase I investigational new drug study (NCT00569231) in which the largest wart was treated with Candin® (Allermed, San Diego, CA), a colorless extract of Candida albicans, our group reported complete resolution of the treated warts in 82% (nine of 11) of the subjects, and complete resolution of distant untreated warts in 75% (six of eight) of the subjects [47] Furthermore, T-cell responses to the HPV 57 L1 peptide were detected in 67% (six of nine) of the complete responders. Because of these immune-enhancing and possible anti-HPV effects of Candin®, the idea of using Candin® as a vaccine adjuvant came about. *In vitro* work performed by Dr. Nakagawa's group showed that Candin® has T-cell proliferative effect, and that the most frequently produced cytokine by LCs exposed to Candin® with and without vaccine peptides was interleukin -12 (IL-12), which promotes T-cell response [30, 48]

1.3.3 Preclinical Safety Data for the Use of HPV Peptides-Candin® Vaccine in Mice

1.3.3.1 Rationale

Although Candin® antigen is FDA-approved for human use, the HPV peptide-Candin® combination has never been tested. HPV 16 E6 protein has been known to mount T-cell immunity in C57BL/6 mice, so we chose this animal model to evaluate safety and immunogenicity of the putative vaccine [49]. Only female mice were examined because the vaccine is being developed for women.

1.3.3.2 Approach

The safety of the HPV peptide-Candin® combination was examined in mice by a **multiple-dose toxicity study (GLP)**. The 25 and 50 μg per peptide doses (corresponding to the two highest doses to be used in the human clinical trial) were used, which were 25 times the human equivalent when adjusted for body surface area. These studies were conducted at the Southern Research Institute.

1.3.3.3 Methods

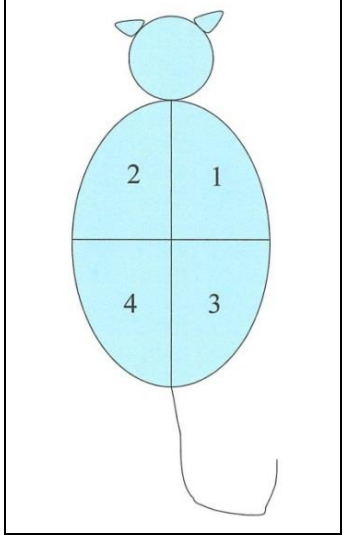
Formulation: Because the CD4 and CD8 T-cell responses to HPV 16 E6 protein are significantly associated with cervical lesion regression [27, 29], the proposed vaccine will consist of four HPV 16 E6 peptides:

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 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 reconstituted with 770 µL of sterile water per vial. After adding Candin®, the mixture was mixed lightly prior to inoculation.

Schedule: Four administrations 3 weeks apart were planned for the Phase I trial; therefore, we performed five administrations in the animal study as recommended, but the frequency was shortened to weekly inoculations (days 1, 8, 15, 22, and 29). The dorsal side of each animal was divided into four areas, and the animals were dosed using a volume of 100 µL/mouse on each dosing day. The dose on each day was split approximately equally between two sites (areas 1 and 2 on days1, 15, and 29; areas 3 and 4 on days 8 and 22) as shown in Fig. 2.

Fig. 2. *Areas of serial injections in mice.*



Route: Intradermal administration.

1.3.3.4 Results

All mice in all dose groups survived to scheduled necropsy. 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 after appearance. Scabs and sore/ulcer at the dosing sites appeared sporadically in all dose 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 peptide and adjuvant administration 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 four 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 consisted of infiltrations of neutrophils, eosinophils, and mononuclear cells. The findings on Day 60 were more chronic in nature. Primarily mononuclear cells were seen with scattered neutrophils and eosinophils. Treatment with the peptides with or without Candin®, and treatment with Candin® alone had no effect on the ability of spleen cells to secrete IFN-γ following overnight stimulation. The only test

article-related macroscopic lesion observed was a crust on Day 32 at the cranial injection site of one animal that was treated with 50 µg of each peptide without Candin®, and one animal that was treated with 50 µg of each peptide with Candin®. In conclusion, the only toxicity observed was transient minimal eosinophil elevation in animals receiving 50-µg peptide with Candin® compared to the vehicle control. This was accompanied by local injection site inflammation (including eosinophil infiltration) on Day 32.

Table 1. Six groups examined in the multiple-dose toxicology study

Group	Treatment	Antigens Dose (µg/mouse)	Adjuvant Dose (µL/mouse)	Total Volume (µL/mouse)	Number of Animals	
					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, sacrificed 3 days after injection. †Recovery, sacrificed 4 weeks after the last injection. F, female.

Table 2. Summary of adverse events from the Phase I clinical trial of PepCan

	CTCAE Grade, Number of Events, (Number of Subjects)															
	Grade 1				Grade 2				Grade 3				Grade 4			
Dose (µg/peptide)	50	100	250	500	50	100	250	500	50	100	250	500	50	100	250	500
Adverse Event																
Injection site reaction, immediate[a]	55 (16)	24 (6)	18 (6)	11 (6)	7 (4)	-	6 (3)	11 (6)	-	-	-	-	-	-	-	-
Injection site reaction, delayed[b]	5 (4)	4 (3)	3 (3)	4 (3)	7 (5)	1 (1)	3 (1)	5 (4)	-	-	-	-	-	-	-	-
Myalgia	23 (9)	4 (1)	4 (1)	4 (3)	-	-	-	1 (1)	-	-	-	-	-	-	-	-
Fatigue	8 (4)	1 (1)	2 (1)	2 (2)	2 (2)	1 (1)	-	-	-	-	-	-	-	-	-	-
Diarrhea	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nausea	11 (6)	5 (3)	-	5 (4)	-	-	-	-	-	-	-	-	-	-	-	-
Vomiting	-	-	-	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-
Headache	10 (4)	3 (3)	5 (2)	6 (2)	3 (3)	-	-	2 (1)	-	-	-	-	-	-	-	-
Pain- body	2 (2)	-	-	-	1 (1)	-	-	2 (1)[u]	-	-	-	-	-	-	-	-
Alopecia	-	-	-	-	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-
Feverish[c]	5 (3)	2 (1)	1(1)	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-
Hot Flashes	-	-	-	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-
Muscle spasm	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Muscle weakness	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flu-like symptoms	6 (3)[u2(2)]	3 (1)	1(1)[u]	-	-	-	1 (1)	-	-	-	-	-	-	-	-	-

	CTCAE Grade, Number of Events, (Number of Subjects)															
	Grade 1				Grade 2				Grade 3				Grade 4			
Dose (μg/peptide)	50	100	250	500	50	100	250	500	50	100	250	500	50	100	250	500
Adverse Event																
Wheezing	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Photophobia	-	-	-	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-
Fracture	-	-	-	-	1 (1)[u]	-	-	-	-	-	-	1 (1)[u]	-	-	-	-
Bruising	-	-	-	-	-	-	-	1 (1)[u]	-	-	-	-	-	-	-	-
Head injury	-	-	-	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-	-
Facial laceration	-	-	-	-	-	-	-	1 (1)[u]	-	-	-	-	-	-	-	-
Allergic reaction	-	-	-	-	-	-	-	1 (1)[u]	-	-	-	-	-	-	-	-
Cholecystitis	-	-	-	-	-	-	-	-	-	-	-	1 (1)[u]	-	-	-	-
Abnormal uterine bleeding	1 (1)[u]	-	-	-	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-
Vaginal infection	-	-	-	-	2 (2)[u]	-	2 (1)[u]	1 (1)[u]	-	-	1(1)[u]	-	-	-	-	-
Vulval infection	-	-	-	-	-	-	1 (1)[u]	-	-	1 (1)[u]	-	-	-	-	-	-
Vaginal irritation	-	-	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-	-	-
Epistaxis	-	-	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-	-	-
Agitation	-	-	1 (1)	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-
Restlessness	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vertigo	-	-	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-	-

	CTCAE Grade, Number of Events, (Number of Subjects)															
	Grade 1				Grade 2				Grade 3				Grade 4			
Dose (μg/peptide)	50	100	250	500	50	100	250	500	50	100	250	500	50	100	250	500
Adverse Event																
Dizziness	1 (1)	-	-	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-
Weight gain	-	-	-	-	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-
Sinusitis	-	-	-	-	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-
Neutropenia	8 (7)[u7(6)]	-	-	2 (2)[u]	-	-	-	-	-	-	-	-	-	-	-	-
Lymphocytosis	-	-	-	-	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-
Thrombocytopenia	-	-	1(1)[u]	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-
Leukopenia	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anemia	6 (6)[u]	-	1(1)[u]	-	-	1 (1)[u]	-	-	-	-	-	-	-	-	-	-
Hypokalemia	9 (9)[u2(2)]	3(3)[u2(2)]	2(2)	2 (2)[u1(1)]	-	-	-	1 (1)	-	-	-	1 (1)[u]	-	-	-	-
Hyponatremia	2 (2)[u]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Serum creatinine increased	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AST increased	1 (1)[u]	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ALT increased	1 (1)[u]	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GGT increased	3 (1)[u]	1 (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bilirubin increased	1 (1)[u]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] appearing < 24 hours from time of vaccination includes site pain, redness, swelling, welt, tenderness, itching. burning, warmness of various grades
[b] appearing ≥ 24 hours from time of vaccination; Includes site pain, redness, swelling, welt, tenderness, itching, burning. warmness of various grades
[c] feeling warm without evidence of temperature ≥ 38.0°
[u] Unrelated adverse event; number of events and subjects presented

1.4 SUMMARY OF PHASE I CLINICAL TRIAL

1.4.1 Methods

A single-arm, open-label, phase I clinical trial was performed to evaluate the safety of PepCan in adult females with biopsy-confirmed HSIL. A dose-escalation phase has been completed in which doses of 50, 100, 250, and 500 μg/peptide/dose (6 subjects/dose level; 4 doses/subject with 3 weeks between each dose) were evaluated to determine the maximum tolerated dose (MTD), the clinically optimal dose (COD), and the immunologically optimal dose (IOD). Blood was drawn for CD3 ELISPOT (to assess CD4 and CD8 responses) and immune suppressor cell analysis before and after the second and fourth injections. HPV-DNA testing was performed before and after the four injections. Clinical response was assessed by performing LEEP excision approximately 3 months after the fourth injection. Subjects who no longer had HSILs were considered to be complete responders, and those with HSILs measuring $\leq 0.2mm^2$ were considered to be partial responders. The final dose phase in which additional 10 subjects were vaccinated at the COD dose of 50 μg/peptide/dose was also performed.

1.4.2 Accrual

At the end of the Phase I study, 52 subjects were enrolled, and 34 received the vaccine. Thirty-one subjects completed the study (mean age of 30.8 ± 6.7 years old).

1.4.3 Safety

One hundred thirty-two injections have been given to 34 subjects. No vaccine-related DLTs were reported. The most common AEs were injection-site reactions both immediate and delayed (**Table 2, Fig. 1**). 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 and delayed 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). In most cases, the injection-site reactions were are easily managed by applying icepacks and topical over-the-counter steroid cream. These reactions do not seem to be delayed-type hypersensitivity reaction, which should appear within 72 hours. Based on the timing of their appearance, these reactions may be manifestation of *de novo* immune stimulation [50].

Other vaccine-related or possibly vaccine-related adverse events, which occurred with ≥ 5% of injections, in the order of decreasing number of occurrences, were myalgia, headache, nausea, fatigue, hypokalemia, feeling feverish, and flu-like symptoms (**Table 2**). None of these adverse events was more than grade 2; these adverse events were self-limiting. One subject experienced asymptomatic hypokalemia requiring treatment with oral replacement potassium (Micro-K 10 mEq per day for 7 days) during the vaccination phase. Her potassium level increased from 3.0 mEq/L to 3.4 mEq/L within 3 weeks, and normalized to 3.8 mEq/L within 3 months.

1.4.4 Clinical response

The histological response rates in order of increasing doses were 50%, 50%, and 33%, and 40% (**Table 3**). 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% [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).

1.4.5 Viral clearance

At least one HPV type present at entry became undetectable in 21 of 31 (70%) patients (**Table 3**). By doses, the rates were 85%, 50%, 50%, and 40% with the highest undetectability at the lowest dose.

1.4.6 Immunological response

1.4.6.1 Systemic

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 (**Table 3**). The lowest response rate was observed in 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 (**Fig. 3**).

Immune profiling (**Fig. 4**, upper panel) showed statistically significant increases in circulating Th1 cells after 2 (p=0.02) and 4 vaccinations (p=0.0004). Th2 cells initially increased significantly (p=0.01) but decreased to below baseline level after 4 vaccinations, although not significantly. Treg levels were minimally changed. The differences in Treg levels prevaccination (p=0.03) and post-2 vaccinations (p=0.04) between these two groups were statistically significant (**Fig. 4**, lower panel).

Fig. 3 *HPV 16 E6- and E7-specific CD3 T-cell responses before vaccinations, after 2 vaccinations, and after 4 vaccinations. T-cell lines were established by stimulating CD3 T-cells with autologous dendritic cells pulsed with HPV 16 E6-vac, E6-GST, E7-vac, and E7-GST. Samples from different visits were tested with overlapping peptides in the same ELISPOT assay, and each region was tested in triplicate. Results are shown for subjects with statistically significant increases to the E6 peptides, and the regions with significant increases (paired t-test) are marked by "*". Subjects 4 and 11 also had a significant increase to E7 (marked by "e"), which may represent the first examples of*

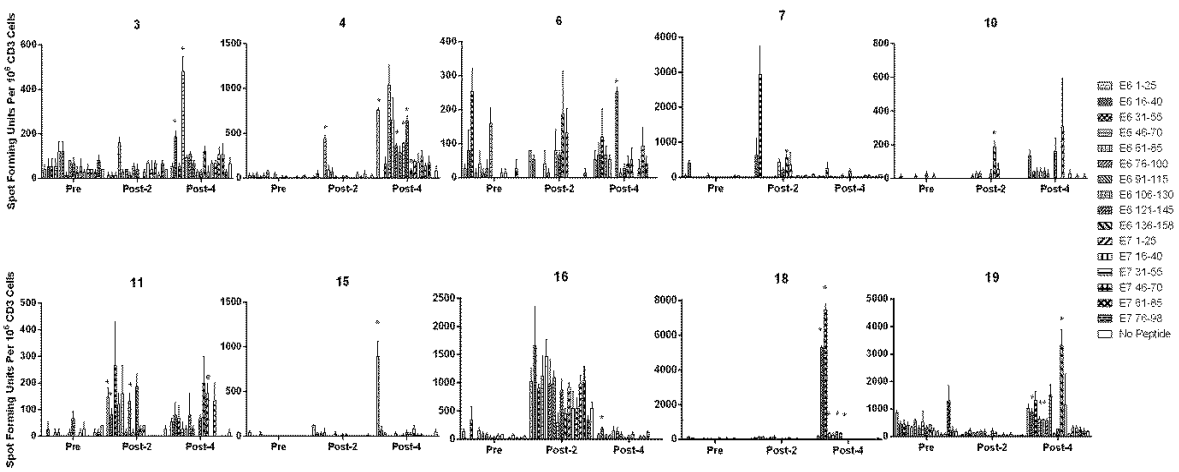
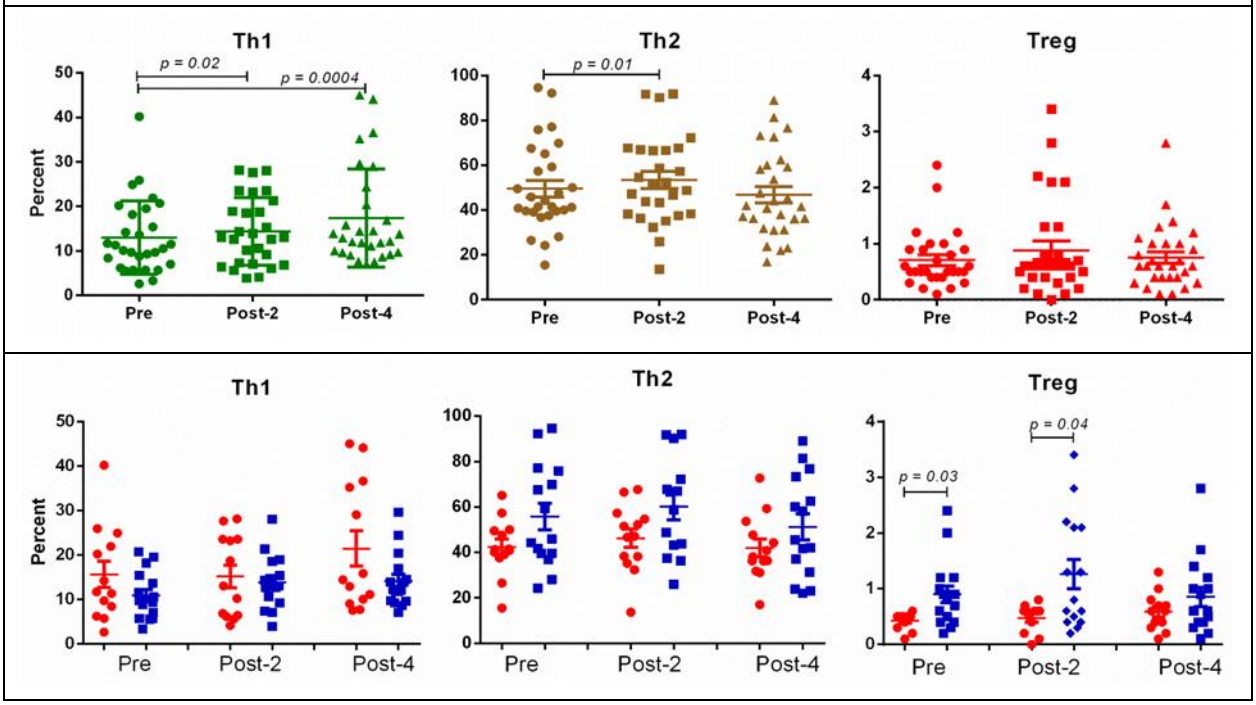


Table 3. Summary of results from the dose-escalation Phase I clinical trial

Characteristics\Dose (n)	50 µg (14)	100 µg (6)	250 µg (6)	500 µg (5)	All (31)
Histological regression, % (n)					
Responders	50 (7)	50 (3)	33 (2)	40 (2)	45 (14)
Virological response[a,b], % (n)					
Responders	85 (11)	50 (3)	50 (3)	40 (2)	70 (21)
Immunological response, % (n)					
New response[c] to HPV-16 E6	64 (9)	67 (4)	83 (5)	20 (1)	61 (19)
Significant response[d] to HPV-16 E6	43 (6)	50 (3)	50 (3)	20 (1)	42 (13)

a At least one HPV type detected at entry became undetectable at exit
b One subject had no HPV detected at entry in the 50 µg group
c New response was detected after vaccination but not prior to initiation of vaccination
d Significant response had $p < 0.05$ using Student's t-test comparing values before and after vaccination

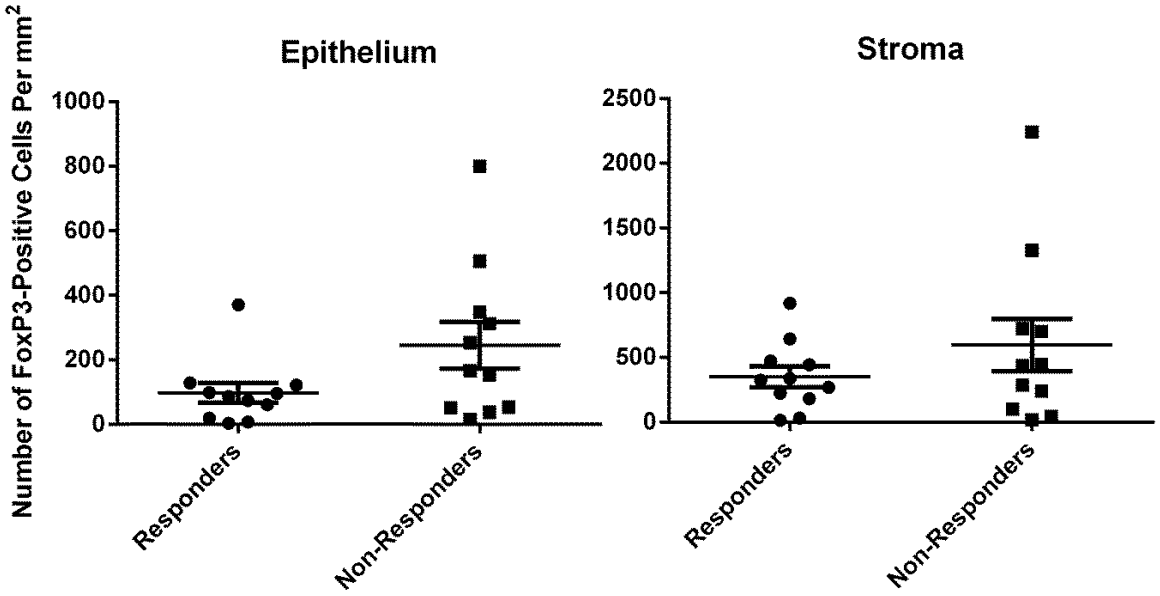
Fig. 4 *FACS analysis of peripheral immune cells. The upper panel show systemic Th1, Th2, and Treg before, after 2, and after 4 vaccinations. In the lower panel, responders are indicated by filled circles while non-responders are indicated by filled squares. None of the subjects with prevaccination Treg 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 Treg cells 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.*



1.4.6.2 Cervical

In the cervix, the number of Tregs were lower in histological responders in the epithelium and the underlying stroma, but not significantly (Fig. 5).

Fig. 5 *Regulatory T-cells in lesional cervical epithelium and the underlying stroma. FoxP3 nuclear staining cells, in lesions (cervical intraepithelial neoplasia 1, 2, and/or3) remaining after vaccination or representative region if no lesions remaining, were counted. The FoxP3 staining cells were also counted in the underlying stroma. The bars represent stand error of means.*



1.5 RATIONALE FOR PHASE II CLINICAL TRIAL

1.5.1 Need for HPV Therapeutic Vaccines

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 [51]. Gardasil, a quadrivalent HPV L1 virus-like particle prophylactic vaccine (HPV types 16, 18, 6, and 11), was the first to be FDA-approved in 2006; a bivalent version (HPV types 16 and 18), Cervarix, was approved by the FDA three years later. Clinical trials have demonstrated excellent vaccine efficacy in women negative for HPV 16 or HPV 18[52, 53], 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 pre-existing HPV infections (n=1,259; 35.5% clearance in vaccinated group, 31.5% in a group receiving a negative control vaccine, p=NS) [51]. Therefore, therapeutic vaccines are needed for cases in which HPV infection is already established and in which HPV-related diseases have already developed. This is the particularly true because the prophylactic vaccine coverage rate in the targeted group (girls aged 13–17 years) has been reported to be only 32% nationally [54]. Although the standard surgical treatments for HSILs such as LEEP are very effective [14], their unintended side effect of increased incidence of preterm delivery from 4.4% to 8.9% [14, 15] has become a concern. Henceforth, the latest guideline no longer recommends treatment for CIN2 in young women (narrowly defined as ≤24 years old and broadly defined as any women who still plans to become pregnant [14]). Treatment is still recommended for CIN3 but observation is now considered acceptable. A new treatment, which does not alter the anatomical integrity of the cervix like the HPV therapeutic vaccine, is very much needed. In short, **HPV therapeutic vaccines are needed** because (1) prophylactic vaccines are not effective against established HPV infection, (2) utilization of the prophylactic vaccines has been low, (3) therapeutic vaccines would leave the cervix intact and would likely not increase the risk of preterm deliveries, and (4) therapeutic vaccine maybe effective against other cancers caused by HPV such as anal, oropharyngeal, penile, vaginal, and vulvar cancers.

1.5.2 Rationale for Proposed Dose of HPV Peptides

In the Phase I clinical trial, four dose levels (50, 100, 250, and 500 μg per peptide) were tested. These four dose levels were chosen based on information available in the literature. Published studies of clinical trials using various peptide vaccines reported using doses that range from 5–3,000 μg per peptide [31-38]. Optimal doses (and smaller doses if two dose levels were the same) for achieving immunogenicity differed greatly among the vaccines: 30 μg of 96-mer malaria peptide [31], 500 μg of 9-mer peptide for treating prostate cancer [34], 50 μg each of 13 HPV 16 E6 and E7 peptides ranging from 25 to 35 amino acids long [35]. Therefore, the dose levels likely to elicit the optimal immunogenicity were chosen.

The dose-escalation portion of the Phase I clinical trial has demonstrated that the 50 μg/peptide/injection was optimal in terms of histological regression, viral clearance, and vaccine-induced immune responses (**Table 3**). Therefore, this dose will be used for the Phase II clinical trial.

1.5.3 Rationale for Proposed Dose of Candin®

Three hundred (300) μL of Candin® will be administered per injection, which was the amount used for intralesional injection of warts [47, 55], as well as the amount of Candin® as a vaccine adjuvant in the Phase I clinical trial. The same amount will be used for the Phase II clinical trial as this amount has been shown to be safe and effective.

1.5.4 Rationale for Proposed Route of Injections

Intradermal route of administration will be used to make use of LCs as antigen-presenting cells. A Phase I clinical trial of a peptide vaccine for prostate cancer administered through this route has shown promising immunogenicity [34]. This route has also been shown to be safe, effective, and immunogenic in the Phase I clinical trial, and will be used for the Phase II clinical trial.

1.5.5 Rationale for Proposed Site of Injections

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 [35, 56]. As injecting in limbs has shown to be safe, effective, and immunogenic in the Phase I clinical trial, the same sites will be used for injection in the Phase II clinical trial.

1.5.6 Rationale for Number of Injections

In published studies of peptide vaccines, the total number of injections ranged from 2 to 17 [31-38]. We proposed to use four injections because Hueman et al. demonstrated that immunogenicity peaked after four injections (six injections in total were given in the study) [34], and four injections appeared to be sufficient in the Phase I clinical trial.

1.5.7 Rationale for Interval between Injections

The interval between injections ranged from 2 weeks to 90 days in the published studies [31-38], but most used a 3-week interval. Kenter and colleagues reported that peptide vaccine immunogenicity measured by IFN-γ ELISPOT assay was less prevalent when blood samples were drawn 7 days after the last vaccination but was higher when they were drawn 3 weeks after the last vaccination [35]. Therefore, we chose the 3-week (± 7 days) interval because it appears to be long enough to allow sufficient mounting of immune responses. As this interval has been shown to be safe, effective, and immunogenic, the same interval will be used in the Phase II clinical trial.

1.5.8 Rationale for Interval between the Last Injection and Final Histologic Assessment

While histological response was assessed 3 months after the last vaccination by performing LEEP in the Phase I clinical trial, the full effect is known to take 1 year [17-19] . In the Phase II clinical trial, PepCan will be administered as an alternative to LEEP, and histological response will be assessed by obtaining colposcopy-guided quadrant biopsies 12 months after the last injection (**Fig. 6**). In a clinical trial which used a similar peptide-based HPV therapeutic vaccine to treat high-grade vulvar intraepithelial lesions, histological regression increased from 25% to 47% between 3 months and 12 months post-vaccinations [18].

1.5.9 Rationale for Primary Outcome Measure: Efficacy

The clinical response to evaluate the vaccine efficacy will be assessed by comparing the punch biopsy results between the Screening Visit (having had HSIL to qualify for vaccination) and the 12-Month Visit (± 2 weeks) (**Fig. 6**). LEEP will not be performed to assess efficacy, but it will be offered at no cost to subjects who have persistent HSILs at the 12 Mo Visit.

The design of the proposed Phase II trial is single-site, and randomized to 2 treatment arms in a double-blinded fashion. We will use a historical placebo group from a clinical trial with similar design (i.e., enrollment of subjects with biopsy-proven CIN2/3, and clinical response assessed by biopsy in 15 month) for comparison [57]. The overall histological regression rate in the dose-escalation Phase I clinical trial was 52% three months after the last vaccination, and this is expected to substantially increase with an extended 12-month observation period.[18] Assuming a conservative rate of 60%, n=35 in the PepCan arm would give 91% power (two-tailed, α=0.05) for detecting a statistically significant difference from the historical placebo group which had a 29% (34 of 117) regression rate [57]. Although there is greater uncertainty regarding the Candin®-only arm, there is ≥90% power to detect a significant differences between the PepCan and Candin® arms under multiple plausible scenarios (for example, regression rates of 67% vs. 29%, or 85% vs. 50%). Forty subjects in each arm will be enrolled to ensure that at least 35 subjects in each would complete the study. While the use of historical placebo group is not as rigorous as having a concurrent placebo group, a concurrent placebo group with biopsy-proven CIN2/3 that would go untreated for 12 months would be difficult to ethically justify.

1.5.10 Rationale for Secondary Outcome Measure: Safety

The combination of HPV peptides and Candin® was first tested in the Phase I clinical trial, and appears to be safe as no DLTs have been reported (**Table 2**). Safety will be assessed in the same manner in the Phase II clinical trial using CTCAE 4.03.

1.5.11 Rationale for Tertiary Outcome Measures: Immunological Response and Viral Clearance

1.5.11.1 Rationale for Measuring HPV-specific T-Cell Response

HPV-specific CD3 T-cell responses will be assessed using immune assay such as the IFN-γ ELISPOT assay before vaccination, after 2 vaccinations, 6 months after 4 vaccinations, and 12 months after 4 vaccinations (**Fig. 6**). In order to evaluate the role of CD3 T-cells in vaccine efficacy, whether clinical response and viral clearance can be predicted based on the CD3 T-cell activities will be assessed.

1.5.11.2 Rationale for Measuring Circulating Immune Cells

The level of circulating immune cells, including Th1 cells, Th2 cells, regulatory T-cells (Treg), and myeloid-derived suppressor cells (MDSC), will be assessed before vaccination, after 2 vaccinations, 6 months after 4 vaccinations, and 12 months after 4 vaccinations. The data from the Phase I clinical trial indicated that PepCan may increase Th1 responses (p=0.02) and decrease Th2

responses resulting in increased effector immune activity (**Fig. 4**). Whether the levels of these circulating immune cells can be used to predict vaccine efficacy in terms of clinical response and viral clearance will be investigated. Vaccine effects on these factors will also be examined throughout the study (i.e., 6 and 12 months instead of 3 months).

1.5.11.3 Rationale for Measuring Viral Clearance

HPV-DNA testing will be performed at the Screening Visit, 6-Month Visit, and 12-Month Visit (**Fig. 6**). In the Phase II study, an HPV type would be considered to be cleared if it is present at the Screening Visit but not at the 6-Month and 12-Month Visits.

1.5.12 Rationale of Other Outcome Measures: Predicting Vaccine Response Using Various Factors Such as Age, Oral Contraceptive Use, Smoking History, Circulating Immune Cells, HLA Types, HPV Types, Cytokine/Chemokine, and Metabolomic Profiling; Evaluating Vaccine Effect; Determining Cross-Protection and Examining Epitope-Spreading and Cross-Reactivity as Possible Mechanisms

Not all vaccine recipients are expected to have clinical response. Some may have persistent HSIL, and some may progress to invasive squamous cell carcinoma. It would be valuable to identify factors that are associated with a favorable response so an educated decision can be made as to who should receive the vaccine.

The Phase I clinical trial has indicated that PepCan is effective in HSILs with HPV 16 and non-16 HPV types. In the Phase II clinical trial, against which non-16 HPV types it is effective may be determined. Furthermore, epitope spreading and cross-reactivity may be investigated as possible mechanisms behind cross-protection.

1.5.13 Rationale for Adding a Candin® Arm

The results of the dose-escalation portion of the Phase I clinical trial showed similar rates of clinical responses in subjects with HSILs associated (4 of 9 or 44%) and not associated (8 of 14 or 57%) with HPV 16 suggesting that *de novo* immune stimulation presumably from Candin® plays a major role. Therefore, Candin® only treatment arm will be added to compare efficacy between PepCan and Candin®.

1.5.14 Rationale for Randomization and Double-Blinding

In order to minimize bias, subjects who are eligible for vaccination will be randomly assigned to one of the two treatment arms (PepCan or Candin®) in a double-blinded fashion so the subjects and study staff (except for pharmacy staff) will not know which treatment is being administered. PepCan and Candin® are both clear solutions prepared in the same 1 mL syringe.

1.6 POTENTIAL BENEFITS AND RISKS TO SUBJECTS

1.6.1 Potential Benefits

The intended benefit is global with the development of a novel HPV therapeutic vaccine for treatment of HSIL preventing the development of cervical cancer. This study will contribute to this goal by evaluating the efficacy and safety of a version of HPV therapeutic vaccine, PepCan.
It is not known whether a particular study subject will benefit, as it is not possible to predict which subjects will qualify for vaccination and of those vaccinated, who will achieve clinical response. However, a subset of subjects who qualify for vaccinations are expected to show clinical response enabling them to avoid a surgical treatment such as LEEP. This would be particularly beneficial to those who plan to conceive after study participation.

1.6.2 Risks of PepCan

No DLTs were observed in the phase I trial (**Table 2**). 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 commonly reported side effects were local swelling, redness, increased skin temperature, and local pain at injection sites.

1.6.3 Strategies to Minimize Risks

All subjects will be screened before enrollment. Thereafter, a pregnancy test will be performed prior to each vaccination, and they will be asked to stay in the clinic for a minimum of 30 min after each injection. To ameliorate possible and anticipated localized minor vaccination site reactions, subjects will be offered a dose of ibuprofen or naproxen after the 30 min observation period. It is generally recommended to start with the lowest dose of these medications after experiencing a prior injection site reaction. However, any dose available can be given.

1.6.4 Strategies to Minimize Bias

The subjects who are eligible for vaccination will be randomized to two treatment groups (PepCan and Candida) at a 1:1 ratio in a double-blinded fashion. A computer generated randomization scheme will be created by a study statistician, which will assign subjects to one of the treatment groups based on the order of first vaccination dates. This information will be forwarded to research pharmacy. A subject will be vaccinated with the same agent for all 4 vaccinations. After the subject completes her 12-Month Visit, a study coordinator will obtain information on which treatment she received from the research pharmacy, and will inform the subject, so she can make informed decisions about her future treatment plans. For a subject who exits the study early for any reason, the treatment she received will be revealed after completing the Early Termination Questionnaire.

2 OBJECTIVES

2.1 PRIMARY OBJECTIVE: EFFICACY

To assess the efficacy of PepCan and Candin® in a Phase II clinical trial by determining clinical response which will be assessed by obtaining colposcopy-guided quadrant biopsies at the 12-Month Visit. Responses will be compared between (1) the PepCan group and a historical placebo group [57], (2) the Candin® group and the same historical placebo group, and (3) the PepCan and Candin® groups. If, upon the 12-Month Visit quadrant biopsies, a subject does not have any evidence of CIN, she would be considered a “complete responder”. If the lesion(s) has(have) regressed to CIN 1, the subject will be considered to be a “partial responder”. If there is still CIN 2 and/or 3 present at the 12-Month Visit, the subject will be considered a “non-responder”. The highest grade among the biopsies will be recorded. In addition to the above analysis of subjects who exited after the 12-Month Visit, another analysis may be performed with addition of subjects who exited the study after the 6-Month Visit if histological results (biopsy and/or LEEP) are available at 6 months.

2.2 SECONDARY OBJECTIVE: SAFETY

Safety will be assessed by documenting AEs from the time of enrollment until the 12-Month Visit according to CTCAE v4.03.

2.3 TERTIARY OBJECTIVES: IMMUNOLOGICAL RESPONSE & VIRAL CLEARANCE

Immunological assessment in terms of HPV-specific CD3 T-cell responses will be assessed using an IFN-γ ELISPOT assay while circulating levels of CD4, Th1, Th2, Treg, and MDSC cells will be assessed by FACS analysis before vaccination, after 2 vaccinations, 6 months after 4 vaccinations, and 12 months after 4 vaccinations. Virological assessments will be made at Screening, 6-Month, and 12-Month Visits.

2.4 OTHER OBJECTIVES

To evaluate predictive factors for response to PepCan or Candin®, various parameters such as age, oral contraceptive use, smoking history, circulating immune cells, HLA types, HPV types, bacterial taxa, cytokine/chemokine, metabolomic profiling, and other factors may be analyzed. Vaccine effects over time on some of these factors will also be assessed.

Cross-protection by PepCan in terms of viral clearance will be determined by tallying each HPV event that is present at Screening Visit but becomes undetectable at both 6-Month and 12-Month Visits for each of the 37 HPV types tested. Viral clearance by *de novo* immune stimulation by Candin® will be determined by tallying each HPV event that is present at Screening Visit but becomes undetectable at both 6-Month and 12-Month Visits for each of the 37 HPV types tested.

Epitope spreading and cross-reactivity may be examined in selected subjects in the PepCan arm.

3 INVESTIGATIONAL PRODUCT

3.1 TEST ARTICLE

3.1.1 HPV Peptides

PepCan will contain four HPV 16 E6 peptides: E6 1-45 (Ac-MHQKRTAMFQDPQERPRKLPQLCT ELQTTIHDIILECVYCKQQLL-NH2), E6 46-80 (Ac-RREVYDFAFRDLCIVYRDGNPYAVCDKC LKFYSKI-NH2), E6 81-115 (Ac-SEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINCQK-NH2), and E6 116-158 (Ac-PLCPEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRETQL-NH2) (US Patent No. 8,652,482). Commercially produced cGMP-grade peptides (CPC Scientific, San Jose, CA) will be examined.

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) except during shipping and immediately prior to use.

The UAMS Research Pharmacy will be responsible for peptide receipt, storage, and preparation prior to vaccination visits.

3.1.2 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.

3.1.3 Combining HPV Peptides and Candin®

Sterile water will be added to a vial containing the four cGMP peptides on the day of use. 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.

3.1.4 Temperature Logs

Daily temperature logs will be maintained by the Pharmacy per standard operating procedures of the Pharmacy. Any deviations in temperature range will be reported to the Sponsor and Principal Investigator.

3.1.5 Drug Accountability Records

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

3.2 TREATMENT REGIMEN

Subjects will receive four injections of PepCan (50 μg/peptide/injection) via intradermal injection in the extremities with three weeks between each injection.

4 STUDY DESIGN

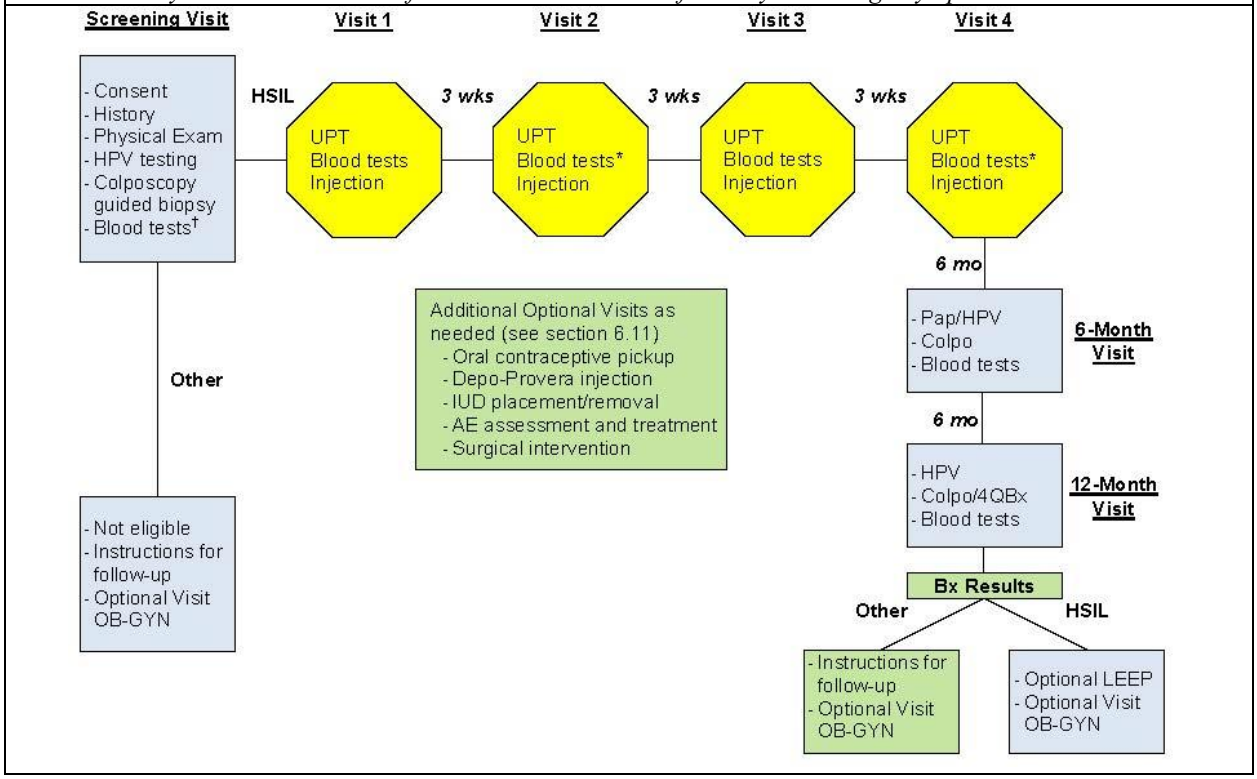
4.1 OVERVIEW

This is a single site Phase II clinical trial of PepCan for treating women with biopsy-proven HSILs randomized and double-blinded to two treatment arms. Half of the subjects will receive PepCan, and the other half will receive Candin® alone. The study design closely resembles the latest guidelines for treating young women with HSIL [14]. Study subjects will be patients attending the UAMS Obstetrics and Gynecology Clinics with untreated biopsy-proven HSILs and patients referred from other clinics. Four injections (one every 3 weeks) of PepCan or Candin® will be intradermally administered in the extremities. Clinical response will be assessed by comparison of colposcopy-guided biopsy results obtained prior to vaccination and at 12-Month Visit. Safety will be monitored from the time of enrollment through the 12-Month Visit. Blood will be drawn for laboratory testing and immunological analyses ("blood test") prior to injection, after the second vaccination, 6 months after the fourth vaccination, and 12 months after the fourth vaccination. Blood will be drawn to aid T-cell analyses ("blood draw") after the first and third vaccinations, and possibly at the Optional Follow-Up and/or Optional LEEP visits. HPV-DNA testing will be performed at Screening and 6- and 12-Month Visits (**Fig. 6**). If a subject has persistent HSIL at the 12-Month Visit or if a subject is withdrawn due to excessive toxicity, she will be given an option to return for a LEEP visit. Alternatively, she may choose to exit the study and be followed by a gynecologist for up to 2 years of observation as recommended before surgical treatment [14].

4.2 RANDOMIZATION TO TREATMENTS

The study randomization schematic will be constructed by the study Statistician. Randomization will occur after Subject eligibility for vaccinations has been confirmed. Subjects will be randomized in a 1:1 ratio to PepCan or Candin® alone. Randomization will be done in book form by Research Pharmacy.

Fig. 6 *Schematic presentation of study visits scheduled for the Phase II clinical trial of our HPV therapeutic vaccine. Colpo, colposcopy, Bx, biopsy, ECC, endocervical curettage, LEEP, loop electrosurgical excision procedure. †These blood tests are for clinical analyses only. *These blood tests are for scientific analyses only. Blood tests may be drawn as needed for clinical and/or scientific analyses during any optional visit.*



4.3 MONITORING TOXICITY

Serious toxicity will be defined (using CTCAE v 4.03) as drug-related:

- Grade II or higher allergic reactions. Grade II is defined as "intervention or infusion interruption indicated; responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics); prophylactic medications indicated for ≤ 24 hours". Grade III is defined as "prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)".
- Grade II or higher autoimmune reactions. 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.

Any subject who experiences serious toxicity will be discontinued from the study.

4.4 INTERRUPTION

In case of prolonged unavailability of vaccine peptides, such as due to failing stability testing and need to manufacture a new lot, all subjects being vaccinated will receive Candin® for the remaining injections. Subjects starting vaccination will be assigned to the Candin® group. After a new lot of peptides become available, randomization will resume with the following possible modifications: (1) the number of subjects to be recruited in the Candin® group will be decreased by the number

of subjects recruited into the Candin® group during the interruption, and (2) the number of subjects in the PepCan group will be increased by the number of subjects who started out receiving PepCan but received Candin® during the interruption. To protect study blinding, UAMS ORRA Quality Assurance will communicate directly with Research Pharmacy and the statistical team. No information regarding which vaccination (PepCan or Candin®) any subjects are receiving will be revealed to the rest of the study team including the Principal Investigator.

4.5 STOPPING RULES

4.5.1 For subject

- A subject should be withdrawn from the study at any point if pelvic examination and histological analysis show evidence of an invasive squamous cell cervical carcinoma or if there is a clinical suspicion of having developed it based on signs and symptoms such as unexplained, prolonged vaginal bleeding. This is to allow the subject to receive the proper work-up and treatment. The subject may proceed to the optional LEEP visit if determined to be beneficial by a study physician. Should a cervical cancer diagnosis be confirmed during the subject's study participation, the subject may receive a hysterectomy if it is determined to be medically necessary.
- 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.

4.5.2 For study

- The study enrollment and vaccine administration 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.
- The sponsor may decide to stop the study at any point, for any reason.

4.6 EMERGENCY UNBLINDING

If a medical emergency necessitating the identity of the vaccine administered occurs, the PI will notify Research Pharmacy and the study Statistician. Research Pharmacy will give identity of vaccine to PI and Study Coordinator and note any incidence in the Randomization book.

5 SUBJECT ENROLLMENT AND STUDY DURATION

5.1 SUBJECT POPULATION, RECRUITMENT, AND INFORMED CONSENT PROCESS

- Women, aged 18 to 50 years, seen at the UAMS Obstetrics and Gynecology Clinics and ANGELS Telecolposcopy program with recent Pap smear results positive for HSIL or "Cannot rule out HSIL" will be recruited through Physician referral, brochures, flyers, UAMS website, letters, phone calls, and word of mouth by study team; interested potential subjects will contact the study coordinator to discuss study; coordinator will conduct initial inclusion/exclusion criteria assessment, schedule subject for screening visit, and send a copy of the informed consent document for the subject to review.
- Other women with recent abnormal Pap smear results positive for HSIL or "Cannot rule out HSIL" will be recruited through clinic referral, brochures, flyers (distributed on and off campus), UAMS website, and advertisements in newspaper, radio, Google ad, and/or social networking site; interested potential subjects will contact the study coordinator to discuss study; coordinator will conduct inclusion/exclusion criteria assessment, schedule subject for

screening visit, and send a copy of the informed consent document for the subject to review; coordinator will request that subject obtain copy of Pap smear result from their physician's office and bring with them to the screening visit.

- Women with recent diagnosis (the duration between the day of diagnosis and the day of 1st injection needs to be ≤ 60 days) of HSIL on colposcopy guided punch biopsy will be recruited through clinic referral, brochures, flyers (distributed on and off campus), UAMS website, and advertisements in newspaper, radio, letters, phone calls, Google ad, and/or social networking site; interested potential subjects will contact the study coordinator to discuss study; coordinator will conduct inclusion/exclusion criteria assessment, schedule subject for screening visit, and send a copy of the informed consent document for the subject to review; coordinator will request that subject obtain copies of medical records of abnormal biopsy from their physician's office and bring it with them to the screening visit.

5.1.1 Inclusion Criteria

- Aged 18-50 years
- Had recent (≤ 60 days) Pap smear result consistent with HSIL or "cannot rule out HSIL" or HSIL on colposcopy guided biopsy
- Untreated for HSIL or "Cannot rule out HSIL"
- Able to provide informed consent
- Willingness and able to comply with the requirements of the protocol

5.1.2 Exclusion Criteria

- History of disease or treatment causing immunosuppression (e.g., cancer, HIV, organ transplant, autoimmune disease)
- Being pregnant or attempting to be pregnant within the period of study participation
- Breast feeding or planning to breast feed within the period of study participation
- Allergy to *Candida* antigen
- History of severe asthma requiring emergency room visit or hospitalization within the past 5 years
- History of invasive squamous cell carcinoma of the cervix
- History of having received PepCan
- If in the opinion of the Principal Investigator or other Investigators, it is not in the best interest of the patient to enter this study

5.1.3 Informed Consent Process

- Potential subjects will be provided the informed consent form before the screening visit and allowed as much time as needed to make decisions regarding study participation.
- The study coordinator/study team member authorized by PI to administer informed consent discussion will discuss the study in detail (including the age-specific standard of care guidelines as periodically released by the American Society of Colposcopy and Cervical Pathology) with the potential subject at any time before the screening visit or at a UAMS Gynecology clinic when she arrives for the screening visit (prior to any study-related procedures), and answer any questions the subject may have about the study; discussions will be conducted in English or in Spanish by Spanish speaking interpreters.
- Should an enrolled subject become pregnant during the study period she will be provided with an informed consent addendum to verify whether or not the subject would agree to the

collection, storage and use of data about the pregnancy, birth and health of the baby. If the subject agrees, they will be asked to fill out an authorization form for release of information to UAMS.

- As consent is an ongoing process, subjects will be asked if they still wish to participate in the study prior to study procedures conducted at each study visit.

5.2 PACE OF ENROLLMENT

During the Phase I study, approximately two thirds of subjects enrolled qualified for vaccination. Taking into account the screen-failure rate and attrition rate (currently about 5% per year), we plan to enroll 125 subjects for screening, and to initiate vaccination in 80 subjects.

5.3 STUDY DURATION

The study duration will be up to 66 months. Each subject is expected to be in the study for approximately 16 months or longer if LEEP is performed.

6 STUDY VISITS

6.1 SCHEDULING STUDY VISITS

The Study Coordinator will schedule study visits (Screening, Vaccination, 6-Month, 12-Month, and Optional LEEP Visits) at the UAMS Obstetrics and Gynecology Clinics and the UAMS Winthrop P. Rockefeller Cancer Institute (WPRCI). The Screening, 6-Month, 12-Month, and Optional LEEP Visits are expected to take approximately 90 minutes. However, they may be longer on busy clinic days. Vaccination Visits are expected to take approximately 60 minutes.

6.2 STUDY VISIT WINDOWS

6.2.1 Between Visits of an Individual Subject

- The first vaccination visit (Visit 1) should be scheduled as soon as possible after all results from the screening visit are available, and subjects are deemed qualified to continue to the vaccination phase of the study, but no later than 60 days after the day punch biopsy was obtained (the screening day for most of the subjects).
- The subsequent vaccination visits (Visits 2-4) should be scheduled 3 weeks ± 7 days apart.
- The 6-Month Visit should be scheduled 6 months ± 2 weeks following Visit 4
- The 12-Month Visit should be scheduled 6 months ± 2 weeks following 6-Month Visit.
- Optional LEEP visit (if subject chooses) should be scheduled as soon as possible after 12-Month Visit or after determining a subject needs to be withdrawn due to serious toxicity.

6.3 STUDY VISIT LOCATIONS

6.3.1 Screening Visit

UAMS Obstetrics and Gynecology Clinics

6.3.2 Vaccination Visits

WPRCI Infusion center 1

6.3.3 6-Month, 12-Month, Optional LEEP Visits

UAMS Obstetrics and Gynecology Clinics

6.3.4 Optional Follow-Up Visits (If necessary)

UAMS Obstetrics and Gynecology Clinics or UAMS WPRCI

6.4 SCREENING VISIT

6.4.1 Procedures for Screening Visit

- Review inclusion/exclusion criteria
- Obtain informed consent (if not previously obtained)
- Have the subject fill out "Subject Contact Information" during the visit
- Have the subject fill out "Screening Visit Questionnaire" during the visit
- Obtain demographic information
- Obtain subject's history
  - Medical history: Be sure to ask for history of previous abnormal Pap smears and how they were treated
  - Drug allergies
  - Concomitant medications
- Perform a physical examination
  - Obtain vital signs
    - Blood pressure (<200/120 mm Hg acceptable)
    - Heart rate (50–120 beats per min acceptable)
    - Respiratory rate (<25 breaths per min acceptable)
    - Temperature (<100.4°F)
    - Weight (no restriction)
- For a subject with child-bearing potential
  - Discuss the risks involved in becoming pregnant while receiving vaccine
  - Ask which birth-control method she will be using 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
  - Ask if subjects need the study to provide birth control and discuss options. All of the options below are available to subjects free of charge while participating in the study.
    - Sprintec is an oral contraceptive and it is available throughout the study.
    - Low-Ogestrel is an oral contraceptive and it is available throughout the study for subjects who need to be taken off Sprintec for medical reasons.
    - Depo-Provera is a contraceptive given as a shot every 3 months and it is available throughout the study.
    - Liletta is an intrauterine device contraceptive and is available only during the first 3 months of participation. An exception would be allowed if an existing IUD were

removed during one of the study visits. In this situation, the IUD may be replaced with Liletta throughout the study.

- The study will cover the cost of the IUD, IUD placement within the first 3 months of study participation (with the exception of replacing an IUD removed during study visits), and IUD removal during your study participation, but not afterwards.

- Perform colposcopy
  - Obtain ThinPrep for HPV-DNA testing
  - Obtain punch biopsy and endocervical curettage if determined to be necessary by the physician (HSIL needs to be confirmed to be eligible)
  - Physician may acquire four-quadrant blind biopsy if no areas of lesions are visible upon colposcopy
  - Record the lesion(s), locations on the cervix, image cervix using the colposcope-mounted image capture system (if available), and indicate where biopsy was taken
  - Record in how many cervical quadrants the lesions are visible
  - If the subject has already been diagnosed with HSIL by biopsy, there is no need to repeat it. However, colposcopy could be repeated to document the location of the lesion(s), and to collect ThinPrep for HPV-DNA and bacterial testing.
  - Colposcopy may be performed in the OR if medically necessary. A COVID-19 test may be required per hospital policy prior to intervention in the OR.
- Draw blood tubes for complete blood count (CBC), and comprehensive metabolic panel (CMP) (to be performed in UAMS clinical laboratory)
  - Comprehensive metabolic panel (CMP) testing
    - Alanine transaminase (no restriction)
    - Aspartate transaminase (no restriction)
    - Albumin (no restriction)
    - Alkaline phosphatase (no restriction)
    - Total bilirubin (no restriction)
    - Total protein (no restriction)
    - Sodium (no restriction)
    - Potassium (no restriction)
    - Chloride (no restriction)
    - CO_2 (no restriction)
    - Blood urea nitrogen (no restriction)
    - Creatinine (no restriction)
    - Calcium (no restriction)
    - Glucose (no restriction)
  - Complete Blood Count (CBC) testing
    - White count (>$3x10^9$/L acceptable)
    - Hemoglobin (>8 g/dL acceptable)
    - Hematocrit (no restriction)
    - Platelet count (>50x109/L acceptable)

6.4.2 Follow-Up to the Screening Visit

The Study Coordinator and Principal Investigator or Co-Investigator will review all information and test results from the screening visit, and will determine whether the subject is eligible to receive vaccination. Eligibility for vaccination includes presence of HSIL or "cannot rule out HSIL" by colposcopy guided biopsy, no evidence of invasive squamous cell carcinoma, vital signs within certain limits, and certain blood tests within acceptable ranges.

- If eligible for vaccination, schedule vaccination visits at WPRCI Infusion center 1
- If not eligible for vaccination, inform subject via phone call, and schedule a follow-up visit if necessary. If a subject has a condition, which requires further medical care, including invasive squamous cell carcinoma, study physicians will refer her to receive appropriate medical care.

6.5 VACCINATION VISITS (VISITS 1-4)

6.5.1 Procedures for Visit 1

- Ask if any medications have been started or stopped since the last visit
- Urine pregnancy test prior to vaccination
- Measure height and weight to determine BMI
- 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 unless pregnant then draw only 2 tubes)
  - CBC (to be performed in UAMS clinical laboratory)
  - CMP (to be performed in UAMS clinical laboratory)
- Administer vaccination injection
- Repeat vital signs after at least 30 min has passed since the injection
- Monitor for any immediate adverse reactions
- Offer dose of ibuprofen or naproxen
- Hand out "Subject Diary" and ask the subject to fill it out and bring it back at the next visit

6.5.2 Procedures for Visit 2

- Ask for the filled out "Subject Diary". If the subject did not return it, ask "Have you experienced any side effects since the last injection?"
- Ask if any medications have been started or stopped since the last visit
- Urine pregnancy test prior to vaccination
- 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 unless pregnant then draw only 2 tubes)
- Administer vaccination injection
- Repeat vital signs after at least 30 min has passed since the injection
- Monitor for any immediate adverse reactions
- Offer dose of ibuprofen or naproxen
- Hand out "Subject Diary" and ask the subject to fill it out and bring it back at the next visit

6.5.3 Procedures for Visit 3

- Ask for the filled out "Subject Diary". If the subject did not return it, ask "Have you experienced any side effects since the last injection?"
- Ask if any medications have been started or stopped since the last visit
- Urine pregnancy test prior to vaccination
- 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 unless pregnant then draw only 2 tubes)
  - CBC (to be performed in UAMS clinical laboratory)
  - CMP (to be performed in UAMS clinical laboratory)
- Administer vaccination injection
- Repeat vital signs after at least 30 min has passed since the injection
- Offer dose of ibuprofen or naproxen
- Monitor for any immediate adverse reactions
- Hand out "Subject Diary" and ask the subject to fill it out and bring it back at the next visit

6.5.4 Procedures for Visit 4

- Ask for the filled out "Subject Diary". If the subject did not return it, ask "Have you experienced any side effects since the last injection?"
- Ask if any medications have been started or stopped since the last visit
- Urine pregnancy test prior to vaccination
- 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 unless pregnant then draw only 2 tubes)
- Administer vaccination injection
- Repeat vital signs after at least 30 min has passed since the injection
- Monitor for any immediate adverse reactions
- Offer dose of ibuprofen or naproxen
- Hand out "Subject Diary" and ask the subject to fill it out and bring it back at the next visit

6.6 Interim Contraceptive Use Reminder Letter

6.6.1 Procedure: Mail the Reminder Letter to the subject after vaccination 4

6.7 6-MONTH VISIT

The 6-Month Visit will be scheduled about six months (± 2 weeks) after Vaccination Visit 4.

6.7.1 Procedures for 6-Month Visit

- Perform a physical examination
  - Obtain vital signs
    - Blood pressure

- Heart rate
- Respiratory rate
- Temperature
- Weight

- Ask if any medications have been started or stopped since last visit

- Perform colposcopy
  - Obtain ThinPrep for Pap smear, HPV-DNA and bacterial testing
  - Record the lesion(s), locations on the cervix, image cervix using the colposcope-mounted image capture system (if available)
  - Record in how many cervical quadrants the lesions are visible
- Colposcopy may be performed in the OR if medically necessary. A COVID-19 test may be required per hospital policy prior to intervention in the OR.
- If determined to be necessary by the physician (ONLY in cases where there is a suspicion of progressive disease), obtain punch biopsy and endocervical curettage
- Blood will be drawn for:
  - Immunomonitoring and other analyses (eight 10.0 mL rubber green top sodium heparin tubes unless pregnant then draw only 2 tubes)
  - CBC (to be performed in UAMS clinical laboratory)
  - CMP (be performed in UAMS clinical laboratory)
- If Pap smear and/or biopsy results are suspicious for or consistent with HSIL and if the subject decides not to return for the 12-Month Visit, the optional LEEP visit may be offered as long as a study physician determines doing so would be beneficial.
- If a cervical cancer diagnosis is confirmed prior to being withdrawn from the study, the subject may be offered a hysterectomy if a study physician determines it to be medically necessary.

6.8 Interim Contraceptive Use Reminder Letter

6.8.1 Procedure: Mail the reminder letter after the 6-month visit

6.9 12-MONTH VISIT

The 12-Month Visit will be scheduled approximately six months (±2 weeks) after the 6-Month Visit.

6.9.1 Procedures for 12-Month Visit

- Perform a physical examination
  - Obtain vital signs
    - Blood pressure
    - Heart rate
    - Respiratory rate
    - Temperature
    - Weight
  - Ask if any medications have been started or stopped since last visit
- Perform colposcopy
  - Obtain ThinPrep for HPV-DNA and bacterial testing

- Record the lesion(s), locations on the cervix, image cervix using the colposcope-mounted image capture system (if available)
- Record in how many cervical quadrants the lesions are visible
- Obtain at least one punch biopsy from each of the 4 quadrants and possibly endocervical curettage (these biopsies will be evaluated by 2 pathologists who are blinded to each other's diagnosis, and consensus will be reached in case of non-concordant initial interpretations)
  - Obtain at least one biopsy from each quadrant with visible lesions
  - In a quadrant without visible lesions, obtain at least one biopsy from each quadrant described to have had HSIL lesions at the Screening Visit
  - In a quadrant without visible lesions and without a record of having had HSIL lesions at the Screening Visit, obtain one blind biopsy

- Colposcopy may be performed in the OR if medically necessary. A COVID-19 test may be required per hospital policy prior to intervention in the OR.
  - ECC is required at 12 month visit if the entire squamocolumnar junction is not visualized
- Blood will be drawn for:
  - Immunomonitoring and other analyses (eight 10.0 mL rubber green top sodium heparin tubes unless pregnant then draw only 2 tubes)
  - CBC (to be performed in UAMS clinical laboratory)
  - CMP (to be performed in UAMS clinical laboratory)
- Have the subject fill out "12-Month Visit Questionnaire" during the visit

6.9.2 Follow-Up to the 12-Month Visit

The Study Coordinator and Principal Investigator or Co-Investigator will review all information and test results from the 12-Month Visit and schedule any optional follow-up visits as soon as possible following this visit.

- If no evidence of HSIL upon biopsy, the subject will complete the study.
- If persistent HSIL is present, the subject may choose either to (1) have LEEP performed as a part of the study or (2) complete the study and be followed by a gynecologist.
- If a cervical cancer diagnosis is confirmed prior to being withdrawn from the study, the subject may be offered a hysterectomy if a study physician determines it to be medically necessary.

6.10 OPTIONAL LEEP VISIT

6.10.1 Procedures for LEEP Visit

- Blood may be drawn from some subjects as explained above for:
  - Immunomonitoring and other analyses (eight 10.0 mL rubber green top sodium heparin tubes)
  - CBC, CMP or other tests as needed (to be performed in UAMS clinical laboratory)
- Perform LEEP biopsy
  - ThinPrep specimen for HPV-DNA and bacterial testing may be obtained
  - Excise visible lesion or, if no visible lesion seen, excise from an area where biopsies positive for HSIL were obtained at the 12-Month Visit
  - LEEP may be performed in the OR if medically necessary A COVID-19 test will be required per hospital policy prior to intervention in the OR.

- If a cervical cancer diagnosis is confirmed prior to being withdrawn from the study, the subject may be offered a hysterectomy if a study physician determines it to be medically necessary.

6.10.2 Follow-Up to the LEEP Visit

The study coordinator will contact the subject and review the LEEP biopsy results (after examined and signed out by hospital pathologist on service) (Record in CRF). In the event of inconclusive LEEP results, a repeat LEEP will be offered if a study physician determines it to be medically necessary. Additional follow-up visits will be scheduled as necessary. Blood may be drawn from some subjects as explained above for immunomonitoring and other analyses (eight 10.0 mL rubber green top sodium heparin tubes).

6.11 OPTIONAL FOLLOW-UP VISITS

6.11.1 When Optional Follow-Up Visits should be scheduled

Since a majority would prefer to receive test results and to have the follow-up plan explained over the phone, most subjects will not be scheduled for a follow-up visit after a screening visit. It would be common to schedule a follow-up visit after an Optional LEEP Visit since a gynecologist may need to examine recovery after LEEP. In rare instances, if a diagnosis of invasive cervical cancer is confirmed, a hysterectomy may be offered if a study physician determines it to be medically necessary prior to withdrawing the subject from the study. Follow-up visits can be scheduled:

- After being informed that the subject is not eligible for vaccinations
- Anytime during study participation
- After completing the Optional LEEP Visit
- After exiting the study without completing all the visits
- For evaluation of AEs
- To obtain contraceptives including:
  - Oral contraceptive pick up
  - Depo Provera injections
  - Placement/removal of IUD
- For diagnostic purposes and surgical intervention deemed medically necessary during the subjects' study participation, including but not limited to, colposcopy or LEEP performed in the OR, hysterectomy due to invasive cervical cancer, cone biopsy or medical imaging such as ultrasound, computed tomography (CT) scan, or magnetic resonance imaging (MRI) scan.
  - Surgical intervention required after the subject's study participation has ended will be the responsibility of the subject.
  - For any procedures performed in the OR, a COVID-19 test may be required per hospital policy prior to intervention. The study will cover the cost of mandatory pre-operative COVID-19 testing while institutional COVID-19 precautions are in effect.

6.11.2 Procedure at the Follow-Up Visit

- Gynecologists may perform a pelvic examination if indicated.
- The study coordinator, a clinic nurse, a study nurse, or one of the investigators may explain the subject's condition
- Directions for following up on the condition may be provided
- Blood may be drawn from some subjects as explained above for:

- Immunomonitoring and other analyses (eight 10.0 mL rubber green top sodium heparin tubes)
- CBC, CMP or other tests for evaluation of the AEs as needed (to be performed in UAMS clinical laboratory)

6.12 IF THE SUBJECT DID NOT COMPLETE THE STUDY

Subjects may not complete the study for a variety of reasons including serious toxicity, non-compliance, lost to follow-up, etc. In these instances, and for subjects who completed at least one vaccination visit, the study coordinator will ensure that subjects complete the "Early Termination Questionnaire" over the phone, via e-mail, or by mail (no stamp will be provided).

7 SUBJECT COMPENSATION

7.1 COMPLETERS AND NON-COMPLETERS, TRAVEL STIPENDS AND ANNUAL LIMITS

7.1.1 Subjects Who Complete the Study

A check or gift cards for $300 will be mailed after the completion of the study (after the 12-Month Visit) to a mailing address provided by the subject.

7.1.2 Subjects Who Do Not Complete the Study

- If a subject is withdrawn due to vaccine-related toxicity (see stopping rules), due to becoming pregnant because contraception failure, or in cases of advancing disease after at least one completed vaccination visit, a check in the amount of $300 will be mailed to an address provided by the subject once the "Early Termination Questionnaire" has been completed.
- For subjects who terminated early but not because of toxicity or due to non-compliance including cases of consequent pregnancies, $50 per visit for each of Visits 1-4, the 6-Month Visit, and 12-Month Visit completed will be mailed if the subject completes the "Early Termination Questionnaire". No compensation will be provided for the Screening Visit, Optional Follow-Up Visit(s), and Optional LEEP Visit.

7.1.3 Travel Stipends

- For subjects travelling more than 50 miles one-way to come for appointments, pre-visit travel stipends will be available.
- Stipends will be calculated and verified (by study staff) based on mileage from point of origin to the appointment location using internet mapping software, such as Google Maps or MapQuest.
- Stipends will be provided in the form of a pre-loaded gift card.
- Stipend amounts:
  - $40 per visit for those travelling > 50 miles but < 100 miles
  - $60 per visit for those travelling ≥ 100 miles but < 150 miles
  - $80 per visit for those travelling ≥ 150 miles but < 200 miles
  - $100 per visit for those travelling ≥ 200 miles

7.1.4 Annual Limit on Gift Cards

The maximum amount of gift cards that can be dispensed to each subject is $600 per calendar year. If more than $600 is owed to a subject (very unlikely to ever happen), the amount exceeding $600 will be paid in the next calendar year.

8 OUTCOME MEASURES

8.1 CLINICAL ASSESSMENTS (UAMS Pathology Laboratory)

Clinical response will be assessed (by Pathologists on service in the Pathology Department) by comparing punch biopsy results from screening (having had HSIL is the inclusion criterion) with the quadrant biopsies performed at the 12-Month visit. The result of the highest grade will be recorded. The subject will be considered a "complete responder" if the 12-Month quadrant biopsies are negative for CIN, a "partial responder" if the show show CIN 1 or a "non-responder" if the biopsy shows HSIL (CIN 2 and/or 3). In addition to the above analysis of subjects who exited after the 12-Month Visit, another analysis may be performed with addition of subjects who exited the study after the 6-Month Visit if histological results (biopsy and/or LEEP) are available at 6 months.

8.2 VIROLOGICAL STUDY (HPV-DNA TESTING (Nakagawa Laboratory) AND BACTERIAL TESTING (University of Chicago Argonne Laboratory)

The ThinPrep samples will be tested for the presence of HPV-DNA. A commercially available kit such as the "Linear Array HPV Genotyping Test" may be used (Roche Molecular Diagnostics, Inc., Alameda, CA). This kit tests for 37 HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108). The human β-globin signal will also be assayed as a positive control for sample adequacy for DNA content from each sample. Positive-control samples (with added HPV plasmid DNA and plasmid-encoded human β-globin gene) and negative-control samples (no HPV plasmid DNA and no human β-globin gene) will be provided by the manufacturer and will be included in each experiment. HPV types 31, 33, 35, 52, 58, and 67 will be considered "HPV 16-Related", additionally HPV types 18, 39, 45, 51, 53, 56, 59, 66, 68, 69, 70, 73, and 82 will be considered "High Risk", and types 6, 11, 40, 42, 54, 61, 62, 71, 72, 81, 83, 84, and CP6108 will be considered "Low Risk" [58]. If samples are still available after HPV testing, other gene sequencing and gene expression studies may be performed for subjects who agreed in the consent for future use of remaining samples.

The virological response will be assessed by comparing HPV-DNA testing results before and after vaccination. The subject will be considered a "clearer" if at least one HPV type(s) present before vaccination becomes undetectable at both 6-Month and 12-Month Visits. Otherwise, a subject will be considered a "persistor" as long as at least one HPV type was detected at baseline.

The ThinPrep samples will also be 16S rRNA DNA tested to assess the nature and the diversity of the cervical microbial community. A commercially available Vaginal Microbiome Genome Mix will be used as a positive control, and a liquid in ThinPrep without cervical sampling will be used as a negative control. Illumina MiSeq platform will be used for sequencing. The presence and abundance of microbiome at the species to phylum levels will be correlated with the vaccine response and other immunological parameters.

8.3 IMMUNOLOGICAL ASSESSMENTS

8.3.1 ELISPOT Assay (Nakagawa Laboratory)

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 interassay variability, all three blood samples (before vaccination, after two vaccinations, and after four vaccinations) 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 and E6-GST. ELISPOT assays will be performed as previously described [28]. We typically examine 10 regions within the HPV 16 E6 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. In order to compare each region before vaccination and after 2 or 4 injections, a t test for paired samples will be performed, as described previously [59]. Therefore, each subject will be assessed in terms of the number of regions with statistically significant increased T-cell responses after two injections or four injections determined by using Student's paired t-test. Remaining CD3 T-cells may be used to assess the recognition of homologous epitopes from other high-risk HPV types, to describe novel epitopes, and/or to assess the endogenous processing of such epitopes.

8.3.2 Measuring Immune Cells

8.3.2.1 Circulating Immune Cells (Nakagawa Laboratory)

A small amount of PBMCs (approximately 3 x 106 cells) from blood draws at Visit 1, Visit 3, and Visit 5 will also be used to monitor levels of circulating immune cells such as Tregs and MDSC to assess whether vaccination may decrease their levels [60]. Flow cytometry will be used to determine the number of CD4+ CD25+ FOXP3+ (Treg) [29] and CD11b+CD14+CD33+IL4R□+HLA-DRint/neg (MDSC) cells [29, 61, 62]. T-bet (Th1), GATA3 (Th2), and/or ROR gammaT (TH17) positive cells may also be examined. The number of circulating immune cells will be determined before vaccination, after two, and after four injections.

8.3.2.2 Cervical Immune Cells (UAMS Experimental Pathology Core)

After routine pathological diagnosis has been made from LEEP sample obtained at the Optional LEEP Visit, additional sections may be examined for cervical immune cells such as those positive for CD3 (T-cell), CD4 (helper T-cell), CD8 (cytotoxic T-cell), CD56 (NK cell), CD1a (Langerhan cells important in antigen presentation), CD20 (B-cell), CD68 (macrophage), FOXP3 (Treg), T-bet (Th1), and MadCAM-1 (addressing involved with T-cell infiltration). Eosinophils (Th2) may also be examined.

8.3.2.3 Others

Additional analyses that may be performed using blood samples to assess vaccine response include antibody production to HPV proteins, cytokine/chemokine, and metabolomic responses (Nakagawa and Metabolon laboratories).

9 DATA ANALYSIS

9.1 ASSESSING EFFICACY

A historical placebo group, from a previously reported study with a similar study design (i.e., enrollment of subjects with biopsy-proven CIN2/3, and clinical response assessed by biopsy in 15 months), will be used for comparison [57]. The strict definition of histological response which only considers "complete responders" to be "responders" will be used. Those with any CIN remaining would be considered as "non-responders" for the purpose of comparing with the historical placebo group. The response rate in PepCan or Candin® recipients who completed the trial after the 12-

Month-Visit will be compared with that of the historical placebo group which was 29.1% (34 of 117) using binomial test. The response rates between the PepCan and Candin® groups will be compared using the Fisher's exact test. See "Rationale for Primary Outcome Measure: Efficacy" (Section 1.5.9) for power analysis and sample size justification.

In addition to the above analysis of subjects who exited after the 12-Month Visit, another analysis may be performed with addition ofsubjects who exited the study after the 6-Month Visit if histological results (biopsy and/or LEEP) are available at 6 months. As 21 patients in the placebo arm were removed from the study at 3 or 6 months for having persistent biopsy-proven CIN3 [57], the response rate of 24.6% (34 of 138) will be used for this comparison.

For an intention-to-treat analysis, all subjects who qualified for vaccination will be included regardless of whether any vaccinations were received. In the historical placebo group, 149 subjects were randomized and qualified for vaccination [57]. Therefore, the placebo response rate for this analysis will be 22.8% (34 of 149).

9.2 ASSESSING SAFETY: SUMMARY OF ADVERSE EVENTS

Subjects who received at least one dose of PepCan or Candin® will be included in safety assessments. Results will be tabulated as shown in Table 2. The type of adverse reactions, the CTCAE grades, and whether the reactions are vaccine-related will be indicated.

9.3 ASSESSING IMMUNOLOGICAL RESPONSE AND VIRAL CLEARANCE

9.3.1 Immunological Response

9.3.1.1 CD3 T-Cell Response to HPV

As described above, a paired t-test for paired samples will be performed in order to compare each region with increased positivity index after 2 or 4 injections compared to pre-vaccination, as shown in Fig. 3 for the PepCan arm. An analogous analysis will be performed for the Candin® arm, and the number of regions with statistically significant increases will be compared between the two treatment arms to elucidate the additive effects of the E6 peptides.

A correlation between CD3 T-cell response to HPV and clinical response will be examined by drawing a contingency table for a number of subjects with at least one region with statistically significant increase to E6 in "responders" and "non-responders" separately for the PepCan and Candin® groups. Fisher's exact test will be used.

9.3.1.2 Circulating Immune Cells

The changes in percentage of circulating immune cells such as Th1, Th2, Treg, and MDSC will be compared after 2, 6 months after 4 vaccinations, and 12 months after 4 vaccinations with baseline as shown in Fig. 4. Paired t-test and one-way ANOVA will be performed to determine statistical significance separately for the PepCan and Candin® groups.

The differences between the percentages of each circulating immune cell types will be compared between the "responders" and the "non-responders" at pre-vaccination, post-2 vaccination, 6 months after post-4 vaccination, and 12 months after post-4 vaccination using Wilcoxon rank-sum test separately for the PepCan and Candin® groups.

9.3.2 Viral Clearance and Microbial Community

HPV-DNA and bacterial testing will performed using Thin-Prep samples from Screening, 6-Month, and 12-Month Visits.

A correlation between clinical response and virological response (at least one HPV type becoming undetectable after vaccination) will be examined by drawing a contingency table for responder vs. non-responders and HPV persistence vs. HPV clearance separately for the PepCan and Candin® groups. Fisher's exact test will be used. The presence and abundance of microbial taxa from species to phylum level will be correlated with clinical response and other immunological parameters.

9.4 FACTORS CONTRIBUTING TO STUDY RECRUITMENT AND RETENTION

Based on data provided in "Screening Visit Questionnaire", "Early Termination Questionnaire", and "12-Month Visit Questionnaire", factors that contribute to subject recruitment and retention may be assessed. The Fisher's exact test will be used to compare factors such as frequent use of Facebook private group, motivation for entering the study, or having young children will be compared between the subjects who are withdrawn from the study early and the subjects who completed the study.

9.5 FACTORS PREDICTING CLINICAL RESPONSE AND VIRAL CLEARANCE

Variables for prediction of vaccine response will be analyzed, first by univariate analyses, and then multivariable analysis with variable selection using lasso[63] with ten-fold cross validation. Computations will be performed in the R and R/Bioconductor[64] environments. Variable selection using lasso will be implemented with the package glmmLasso, while enrichment analysis for Gene Ontology terms will be performed using topGO.

10 DATA AND SAFETY MONITORING PLAN

The PI will have the overall responsibility for assuring safety and gathering the data for with assistance from the co-investigators, sub-investigators, and research staff, under the guidance of the Institutional Review Board (IRB). As the sponsor, UAMS is responsible for providing quality monitoring for this study.

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

Monitoring specialists from ORRA will conduct periodic on-site, comprehensive monitoring as determined by a protocol-specific monitoring plan, which will be provided by the ORRA Monitoring Unit.

10.1 DEFINITIONS

10.1.1 Adverse Event

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom, or disease that is temporally associated with the use of the vaccine, and it will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03. Local and/or systemic adverse events may include itching, burning, pain, peeling, rash, oozing, redness, tenderness, scarring, fever, nausea, dizziness, and wheezing. The subjects will be allowed to use and provided analgesics (such as ibuprofen or naproxen) according to the appropriate dosages after injections to limit any adverse events that may occur. Any adverse event will be reviewed and considered related or not related to the vaccine. All applicable events will be reported to the IRB according to IRB policy 10.2 and the FDA according to 21 CFR 312.32.

10.1.2 Serious Adverse Event

A serious adverse event is any medical event that:

- Results in death
- Is an immediate threat to life
- Requires hospitalization or prolongation of existing hospitalization
- Is a congenital anomaly or birth defect, or
- Other important medical events that have not resulted in death, are not life-threatening, or do not require hospitalization, may be considered serious adverse events when, based upon the appropriate medical judgment, they are considered to jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

10.2 ADVERSE EVENTS MONITORING

10.2.1 Time Period

Adverse events will be collected from the time of enrollment until the 12-Month Visit.

10.2.2 Collecting Procedure

Adverse events may be uncovered through any of these methods:

- Observing the subject
- Asking the subject to keep "Subject Diary"
- Receiving an unsolicited complaint from the subject

All adverse events will be recorded in either CRF and/or Communication Note as appropriate. In addition, all adverse events will be recorded in AERS.

10.2.3 Relationship to the Investigational Drug

The relationship between the adverse event and the investigational drug should be assessed using the following categories:

- **Definitely Related:** A direct cause and effect relationship between the investigational drug and the adverse event exists.
- **Possibly Related:** A direct cause and effect relationship between the investigational drug and the adverse event has not been clearly demonstrated, but is likely or very likely.
- **Unlikely Related:** A direct cause and effect relationship between the investigational drug and the adverse event is improbably, but not impossible.
- **Unrelated:** The adverse event is definitely not associated with the investigational drug.

10.3 REPORTING ADVERSE EVENTS

10.3.1 Standard Reporting

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

10.3.2 Expedited Reporting

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

- The UAMS Research Support Center-Regulatory Affairs within 24 hours of PI being notified
- The FDA will be notified using the MedWatch Form 3500A within 10 days of PI being notified (http://www.fda.gov/medwatch/safety/FDA-500A Fillable.pdf)
- The UAMS IRB will be notified of events requiring expedited reporting within 10 days of PI being notified (see below if SAE is death)
- A drug-related death occurring while a subject is on the study must be reported to:
    - The UAMS IRB immediately
    - 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.

11 PROTOCOL DEVIATION AND PROTOCOL VIOLATION

11.1 DEFINITIONS

11.1.1 Protocol Deviation

A study event that is not covered under the existing protocol and represents a failure to comply with the protocol. Most deviations are minor and involuntary. If the deviations represent a variation from the approved protocol that could affect the safety and welfare of the subject, it must be reported to the UAMS IRB immediately.

Missing subject diaries will not be considered protocol deviations, as there are mechanisms in place to collect this information.

11.1.2 Protocol Violation

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.

11.2 REPORTING PROTOCOL DEVIATIONS AND PROTOCOL VIOLATIONS

11.2.1 Standard Reporting

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.

11.2.2 Expedited Reporting

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.

12 ETHICAL CONSIDERATIONS AND REGULATORY COMPLIANCE

12.1 HUMAN SUBJECT PROTECTION

This study will be conducted in compliance with the protocol and with all applicable regulatory requirements.

12.2 UAMS IRB AND PROTOCOL REVIEW AND MONITORING COMMITTEE (PRMC)

- A copy of the protocol and informed consent documents will be approved by the UAMS IRB and PRMC prior to initiation of the study.
- The investigator must submit and obtain approval for any changes in the protocol or informed consent forms. The UAMS Research Support Center must also be notified and provided the revised documents.
- Annual status report will be submitted.
- Change of PI will be notified within 30 days.
- PI will sign a statement regarding the protection of human subjects and vulnerable population in CLARA.

12.3 INFORMED CONSENT

- Before a subject's participation in the trial, the investigator is responsible for obtaining written informed consent from the subject or the subject's legally acceptable representative.
- Before signing the consent, the subject must have received adequate explanation of the objectives, methods, anticipated benefits, and potential risks associated with the study, including age-specific standard of care guidelines as periodically released by the American Society of Colposcopy and Cervical Pathology.
- No study-related procedures are to be performed before the subject has given his/her written informed consent.
- The consent process must be recorded in the Informed Consent Process Note.
- A copy of the signed, written informed consent will be given to the subject.
- Original consent documents will be kept with the study record.

12.4 STUDY DOCUMENTATION AND STORAGE

The investigator shall maintain a list of appropriately qualified persons to whom she 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. A subject's file should include:

- CRFs
- Informed consent documentation
- Source documentation

The study file should contain:

- The protocol and all amendments
- Current curriculum vitas of investigators
- Medical licenses of investigators
- The IRB statement of compliance and membership rosters
- Completed FDA form 1572
- All correspondence to and from the UAMS IRB, PRMC, and FDA.
- Any other study related documents

Study records will be retained on-site in accordance with applicable institutional and federal regulatory requirements.

12.5 STUDY REGISTRATION AND PUBLICATIONS

This clinical trial will be registered at ClinicalTrials.gov (www.clinicaltrials.gov), and information will be updated in a timely manner. The findings from this study will be presented at professional society meetings at national and international levels, and will be published in peer-reviewed journals.

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14 APPENDIX

14.1 Appendix 1. Schedule of Study Assessments

	Within 60 days of Visit 1	Visits scheduled 3 wks (± 7 days) apart				6 months (± 2 wks) from Visit 4	6 months (± 2 wks) from 6-Month Visit	As soon as possible following 6-Month or 12-Month Visit	Additional Optional Follow-Up Visit(s)
	Screening Visit	Visit 1	Visit 2	Visit 3	Visit 4	6-Month Visit	12-Month Visit	Optional LEEP Visit	
Informed Consent	X								
Inclusion/ Exclusion Review	X								
Screening Visit Questionnaire	X								
Subject Contact Information	X								
History	X								
Physical Exam[a]	X					X	X		
Colposcopy[bc]	X					X	X		
ThinPrep sample[d]	X					X	X	X[e]	
Complete blood count (CBC)[f]	X	X		X		X	X	X[g]	X[g]
Comprehensive metabolic panel (CMP)[h]	X	X		X		X	X	X[g]	X[g]
Blood for Immunological Assessments[i]		X	X	X	X	X	X	X[g]	X[g]
Urine Pregnancy Test		X	X	X	X				
BMI		X							
Pre-Injection Vital Signs		X	X	X	X				
Vaccination		X	X	X	X				
Post-Injection Vital Signs		X	X	X	X				
Adverse Events		X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X		
Analgesics offered		X	X	X	X				
Provide Subject Diary		X	X	X	X				
Collect Subject Diary[j]			X	X	X	X			
12-Month Visit Questionnaire							X		
Optional LEEP[jc]								X	
Hysterectomy[k]						X	X	X	X
Visit Follow-up[l,m]	X							X	X[n]

[a] Physical Examination will include blood pressure, heart rate, respiratory rate, body temperature, and body weight.
[b] Colposcopy will include punch biopsy and endocervical curettage as indicated; biopsies will be obtained from all subjects at the 12-Month visit.
[c] If performed in the OR, a COVID-19 test may be required per hospital policy prior to intervention. The study will pay for the mandatory pre-operative COVID-19 testing while institutional COVID-19 precautions are in effect.
[d] Specimens will be collected for HPV-DNA and bacterial testing at Screening, 6- & 12-Month Visits. Pap smear added only at the 6-Month Visit.
[e] This only needs to be collected when requested.
[f] CBC will include white count, hemoglobin, hematocrit, and platelet counts.
[g] These bloods draws only need to be done when requested.
[h] CMP will include aspartate transaminase, alanine transaminase, albumin, alkaline phosphatase, total bilirubin, total protein, sodium, potassium, chloride, CO2, blood urea nitrogen (BUN), creatinine, calcium, and glucose
[i] Blood samples will be collected for ELISPOT assay, circulating immune cells, and/or other research laboratory assessments.
[j] It is anticipated some participants in this population will not return completed diaries. However, there are mechanisms in place to collect this information. When this occurs staff, will ask subjects whether they have experienced any adverse events or changes to concomitant medications and document their response in Epic. Missing diaries will not be considered protocol deviations.
[k] In rare instances, should a diagnosis of invasive cervical cancer be confirmed, a hysterectomy may be offered if a study physician determines it to be medically necessary. This should be scheduled as soon as possible following the determination of medical necessity.
[l] Test results and visit outcomes will be discussed with subjects via telephone. Optional follow-up visits may be scheduled to discuss test results, perform clinically indicated examinations/procedures, and/or further discuss a subject's condition. These should be scheduled as soon as possible.
[m] Based on the results of the ELISPOT assay, some subjects will be furthered studied for cross-reactivity, epitope spreading, and/or defining novel T-cell epitopes.
[n] Other reasons for additional optional follow-up visits during study participation are referenced in section 6.11.