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A Pilot Study of pNGVL4a-CRT/E7(detox) for the Treatment of Patients with HPV16+ Cervical Intraepithelial Neoplasia 2/3 (CIN2/3)

Ronald D. Alvarez, M.D., M.B.A.¹, Warner K. Huh, M.D.¹, Sejong Bae, Ph.D.¹, Lawrence S. Lamb Jr., Ph.D.¹, Michael G. Conner, M.D.¹, Jean Boyer, Ph.D.², Chenguang Wang, Ph.D.³, Chien-Fu Hung, Ph.D.³, Elizabeth Sauter³, Mihaela Paradis³, Emily A. Adams, MB(ASCP)CM³, Shirley Hester, R.N.¹, Bradford E. Jackson, Ph.D.¹, T.C. Wu, Ph.D.³, and Cornelia Trimble, M.D.³

¹University of Alabama at Birmingham

²University of Pennsylvania

³Johns Hopkins University School of Medicine

Abstract

Objective—The purpose of this study was to evaluate the safety, efficacy, and immunogenicity of a plasmid vaccine, pNGVL4a-CRT-E7(detox), administered either intradermally, intramuscularly, or directly into the cervical lesion, in patients with HPV16-associated CIN2/3.

Methods—Eligible patients with HPV16⁺ CIN2/3 were enrolled in treatment cohorts evaluating pNGVL4a-CRT-E7(detox), administered by either particle-mediated epidermal delivery (PMED), intramuscular injection (IM), or cervical intralesional injection, at study weeks 0, 4, and 8. Patients were monitored for local injection site and systemic toxicity. A standard therapeutic resection was performed at week 15. The primary endpoints were safety and tolerability. Secondary endpoints included histologic regression and change in cervical HPV viral load. Exploratory endpoints included immune responses in the blood and in the target tissue.

Results—Thirty-two patients with HPV16⁺ CIN2/3 were enrolled onto the treatment phase of the study, and were vaccinated. Twenty-two of 32 patients (69%) experienced vaccine-specific related adverse events. The most frequent vaccine-related events were constitutional and local

Address correspondence to: Ronald D. Alvarez, M.D., M.B.A., 176F RM 10250, 619 19th St S, Birmingham, AL 35249-7333, rdalvarez@uab.edu, Phone: 205-934-4986, Fax: 205-975-6174.

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Conflicts of Interest

Dr. T-C Wu is a co-founder of and has equity interest in Papivax, LLC and owns Papivax Biotech Inc. stock options and is a member of their Scientific Advisory Board. Under a license agreement between Papivax Biotech, Inc., and the Johns Hopkins University, Dr. Wu is entitled to royalties on the vaccine described in this article. This arrangement has been approved by the Johns Hopkins University in accordance with its conflict of interest policies.

Dr. Warner K Huh serves as a compensated consultant for Thevax and Merck. Neither of these companies have a corporate interest in the vaccine described in this article.

None of the other authors have any conflicts of interests to report.

injection site in nature, and were grade 1 or less in severity. Histologic regression to CIN 1 or less occurred in 8 of 27 (30%) patients who received all vaccinations and underwent LEEP. In subject-matched comparisons, intraepithelial CD8+ T cell infiltrates increased after vaccination in subjects in the intralesional administration cohort.

Conclusion—pNGVL4a-CRT-E7(detox) was well-tolerated, elicited the most robust immune response when administered intralesionally, and demonstrated preliminary evidence of potential clinical efficacy.

Introduction

Cervical cancer remains one of the leading causes of cancer death in women worldwide, particularly in low-resource areas. Prophylactic vaccines that prevent infection with HPV types 16 and 18, the genotypes most commonly associated with invasive cancers, have proven to be effective. [1,2] Yet, high grade cervical intraepithelial neoplasia (CIN2/3), a known precursor to invasive cervical cancer, remains very common, even in high-resource settings, and is likely to remain so for decades.[3] Presently, the standard therapies for CIN2/3 are either excisional or ablative. These treatments are destructive and can be associated with acute adverse side effects and long-term reproductive morbidity. [4] Moreover, recurrences are not uncommon, particularly in patients with involved margins in the surgical specimen, and those with risk factors for cervical dysplasia. There is an increasing frequency of viral integration in HPV16-associated preneoplastic lesions. [5,6] The severity of preneoplastic lesions is associated with integration of the HPV viral genome into the host genome, and subsequent constitutive expression of two viral proteins, E6 and E7. As such, expression of both of these non-‘self’ proteins is functionally required for disease initiation and persistence in invasive cervical cancers and their precursor lesions; thus, they are thereby compelling, ubiquitous immunotherapeutic targets. [7,8]

Ongoing research efforts have been devoted to the development of HPV vaccines for patients with established CIN2/3 and invasive cervical cancer. We have developed a novel therapeutic vaccine, pNGVL4a-CRT/E7(detox), which targets HPV16 E7.[9] This DNA vaccine is comprised of a pNGVL4a expression vector containing coding sequences for HPV16 E7 linked to calreticulin (CRT). The E7 sequence in this construct has been modified at aa24 and 27, which abrogates its transforming potential. In preclinical models, CD8+ T cells elicited by this construct demonstrate antitumor effect against epithelial cells expressing wildtype E7. The advantage of using the entire E7 sequence is that determinant selection occurs in the host and is not limited by HLA Class I allele. Calreticulin is a 46 kDa calcium-binding chaperonin related to the family of heat shock proteins (HSPs). It promotes assembly of MHC I-peptide complexes delivered into the endoplasmic reticulum by transporters associated with antigen processing (TAP-1 and TAP-2), and also complexes with MHC class I-β2m molecules to aid in antigen presentation. Moreover, CRT and its protein fragment (aa 1-180), vasostatin, have been shown to selectively inhibit vascular endothelial cell proliferation, and to suppress tumor growth. In sum, this vaccine has been designed to potentially exploit both the effect of enhancing antigen-specific MHC class I presentation as well as eliciting targeted local anti-angiogenesis.

Preclinical studies have validated the potential of pNGVL4a-CRT/E7(detox) as both a preventive and therapeutic vaccine approach for HPV related tumors. [9] Transfection of 293 D^bK^b cells with CRT linked to E7 demonstrated more efficient localization of the E7 antigen to the endoplasmic reticulum compared to that noted in cells transfected with E7 alone, thus enhancing E7 antigen presentation. Vaccination of mice with CRT fused to E7 induced significant increases in E7-specific CD8 T cell precursors in splenocytes and an increase in serum E7-specific antibody titers compared to mice vaccinated with E7-DNA alone. In an *in vivo* cervical cancer model system, TC-1, vaccination with chimeric CRT/E7 DNA in C57BL/6 mice elicits both an enhanced preventive effect when vaccination is performed prior to tumor challenge, as well as anti-tumor effect in mice vaccinated after tumor engraftment, compared to mice vaccinated with E7-DNA. Additional qualitative and quantitative studies demonstrate that this anti-tumor efficacy model is mediated by CD8 T cell responses to E7, but also in part by an anti-angiogenic effect.

We sought to translate these encouraging preclinical findings into the context of a first-in-human clinical trial. The purpose of this study was to evaluate the safety and feasibility of pNGVL4a-CRT/E7(detox) vaccination administered via various routes to patients with HPV16⁺ CIN2/3, and to determine the clinical and immunologic response to this therapeutic HPV vaccine approach.

Methods

Study Vaccine

Clinical grade vaccine, pNGVL4a-CRT/E7(detox), was manufactured under Good Manufacturing Practices by the National Cancer Institute Rapid Access to Interventional Development (RAID) program, and met all acceptance criteria for release. (RAID ID# 471) GMP grade vaccine was manufactured at the Biological Resources NCI branch at Frederick, Maryland, and was formulated for intramuscular delivery by the NCI and for epidermal delivery by Powdermed. (Leicestershire, UK)

Patient Eligibility

Patients were recruited from the colposcopy clinics at the University of Alabama at Birmingham (UAB) and at Johns Hopkins University (JHU). Potential patients participated in a screening phase to determine eligibility prior to being enrolled into a vaccine administration phase. To be eligible for this study, female patients age 19 years or greater were required to have HPV16-positive (HPV16+) CIN2/3 confirmed by colposcopy and biopsy. HPV16 expression was assessed by the Hopkins Molecular Pathology Core Lab, using the HPV16-specific TaqMan kinetic PCR method developed by Gravitt et al. [10] Patients were required to have a measurable lesion after biopsy. Patients were required to have a hemoglobin of 9 g/dl or greater and to be both HIV and hepatitis B seronegative. Patients with cytologic or histologic evidence of glandular dysplasia were not eligible for this study. Patients who were pregnant, had active autoimmune disease, were taking immunosuppressive medication, or had history of arterial or venous thrombosis were not eligible for this study. Patients with an allergy to gold or prior chrysotherapy were also ineligible. All patients were required to provide informed consent for the screening phase

and, if eligible, for the administrative phase. Prior vaccination with a VLP based HPV vaccine was permitted. Institutional Review Board approval was obtained at both institutions prior to the initiation of the trial (NCT00988559).

Trial Design

This study was a pilot translational trial evaluating escalating dosages of the HPV16 E7-targeted DNA vaccine pNGVL4a-CRT-E7(detox) administered in cohorts of up to 6 eligible study patients (Table 1). The vaccine was administered by one of three routes: 1) particle-mediated epidermal delivery (PMED) in the thigh, using a needle-free ND10 delivery system developed by PowderMed, Ltd.; 2) IM delivery in the deltoid muscle; or 3) direct intralesional/intramucosal delivery in the cervix. The ND10 PMED delivery device is closely related to the ND5.5 PMED delivery device used in a prior phase I study, but has a reduced number of components to ease large-scale manufacturability. Like the ND5.5 it uses pressurized helium from an internal cylinder to accelerate gold particles of 1–3 μm diameter coated with DNA into the epidermis. [11] ND10 devices were formulated to contain either 2 μg pPML7789 per 1 mg of gold particles (designated H5) or 1.8 μg pPML7789 plus 0.2 μg pPJV2012 per 1 mg gold particles (designated H5/DEI-LT).

The vaccine was administered at study weeks 0, 4, and 8. A loop electrosurgical excisional procedure (LEEP) or cold knife conization was performed seven weeks after the third vaccination, at study week 15. The overall goals of the study were to assess: 1) the safety and feasibility of vaccine administration, 2) the clinical response, and 3) the induction of an immune response to the vaccine antigen. Primary endpoints included standard safety and tolerability endpoints as defined in CTCAE v4.0. Secondary and exploratory endpoints included histologic regression, defined as no CIN2/3 in the resection specimen, cervical viral load, and immune response to vaccine antigen. Immunogenicity was assessed by HPV16 E6/E7-specific IFN- γ ELISpot assays with cryopreserved PBMCs obtained at screening (t0), at study week 8-10 (t2), at study week 15 (t3), and at study week 19 (t4).

Toxicity and Dose Modification

Patients were assessed for adverse effects by history and physical prior to and at weeks 4, 8, 15, 19, 28, and 41. Patients were also given diary cards to record symptoms. Toxicity assessment primarily focused on local injection site reactions, and solicited general signs and symptoms including allergic reaction, fever, and cytokine release syndrome. These as well as other toxicities were designated utilizing Common Toxicity Criteria V 4.0. No clinical laboratory testing was performed after vaccination. All adverse events that occurred from the time of study drug administration through 30 days after the final treatment were recorded and grade and attribution were appropriately assigned. Dose-limiting toxicity was defined as any grade 3 toxicity in any organ system or grade 2 or higher allergic reaction/hypersensitivity reaction. Subjects who became pregnant during study were required to discontinue vaccination. Subjects removed from the study were replaced in the respective treatment cohort.

Histologic evaluation

Patients underwent colposcopy at baseline (pretreatment), at week 8. A standard therapeutic resection of the cervical squamocolumnar junction (either a cold knife conization or a LEEP) was performed at study week 15, seven weeks after the third vaccination. Routine histologic diagnoses were made on H&E sections, and reviewed subsequently by study pathologists blinded to treatment group. Histologic regression was defined as CIN 1 or less in week 15 resection specimen.

HPV viral load

HPV 16 viral load was measured in exfoliated cell specimens obtained pretreatment and at weeks 8 and 15, by the Hopkins Molecular Pathology Core Lab, using the HPV16-specific TaqMan kinetic PCR method developed by Gravitt et al. [10]

Immune responses to vaccine antigen

ELISpot assays were performed by the University of Pennsylvania Human Immunology Core Facility using a qualified protocol as described previously. [12] The standard ELISpot protocol with 24-hour peptide stimulation was previously cross-validated across different laboratories. [13] Two previously described groups of 15-amino acid residues spanning the length of HPV16 E6 and E7, respectively, were pooled at a concentration of 2 mg/mL per peptide. [14, 15] For each time point, the mean number of SFU from triplicate wells with PBMCs incubated with medium alone (background) were subtracted from the means of PBMCs stimulated with HPV16 E6 or E7 peptides. After subtracting medium control, a positive response was defined as at least 20 SFU/10⁶ PBMCs and greater than two times the SD of the pre-vaccination antigen-specific response.

Immunohistochemical staining

Tissue in paraffin blocks that was residual after histopathologic diagnoses had been finalized were used for immunohistochemical (IHC) staining for CD8. Five-micron sections were cut from formalin-fixed, paraffin-embedded tissue from the pre-vaccination (diagnostic biopsy) and from the post-vaccination resection (7 weeks after the third vaccination). Heat-based antigen retrieval was performed for 30 minutes, followed by blocking endogenous peroxidase with 0.3% H₂O₂, and incubation with primary antibody. The primary antibody was detected using the PowerVision+ Poly-HRP IHC detection system (Leica Biosystems), as per the manufacturer's instructions. After incubation with detection reagents, Diaminobenzidine was used as a chromogen, and Harris hematoxylin was used as a counterstain. The primary antibody used for CD8+ was clone 4B11 (Leica Biosystems, dilution 1:500, incubation overnight at 4°C).

Quantitation of tissue immune cells in histologic sections

Images were captured using a Nikon E600 microscope/Plan fluor ×20/0.50 ocular and a DXM1200f Nikon digital camera (Nikon, Melville, NY), as described previously. [16] Regions of interest (ROIs) were delineated in normal epithelium, in stroma immediately beneath normal epithelium, in CIN2/3 epithelium, and in stroma immediately subjacent to CIN2/3, using NIS Elements AR3 imaging software (Nikon). The intensity of chromogen

infiltrates was quantitated by normalization against the area of the ROI (μm^2). At least 3 and up to 10 discrete ROIs were quantified in each compartment in each case. The mean density of chromagen staining for each compartment for each case was calculated and used for statistical comparisons between groups.

Statistical Design and Analysis

This was a pilot study to evaluate the safety of pNGVL4a-CRT-E7 (detox) immunization at two dose levels using three different routes of administration. Sample size was based on the traditional 3+3 design with six dosing cohorts. If no DLTs were observed at the low dose levels for each route of vaccination, then we proceeded with accrual to the higher dose level. If a DLT was experienced by 1 of 3 subjects in a dose cohort, then up to 3 additional subjects could be accrued to that treatment group. If a DLT was experienced by 2 or more subjects in a dose cohort, then accrual of additional patients would have ceased. The maximum sample size for this study was up to 36 patients.

The demographics and baseline characteristics for patients enrolled onto the study were tabulated and summarized for each route of delivery using descriptive statistics (mean, median, standard deviation, range, frequency and percentage). The detailed properties and severity of reported vaccine-specific adverse events were tabulated, and frequencies and percentages of events assessed for each route of delivery. The clinical responses were tabulated and frequencies and percentages of events were presented for each route of delivery. Non-parametric Wilcoxon signed rank tests were conducted for evaluating the changes in HPV viral load pre- and post-vaccination and the immune responses. Kruskal-Wallis rank sum tests were conducted for comparisons of the different route of delivery cohorts.

Results

Patient Characteristics

A total of 105 patients (99 at UAB, 6 at JHU) were enrolled onto the screening phase of the study between November of 2009 and September of 2013. One patient had a positive pregnancy test and was not tested for HPV. Sixty-five patients tested negative for HPV16. Four patients tested positive for HPV16 but elected not to enroll on the treatment phase of the study. Three patients tested positive for HPV16, agreed to enroll on the treatment phase of the study, but were lost to follow up and did not receive vaccination. Thirty-two patients (30 at UAB, 2 at JHU) tested positive for HPV16, were enrolled onto the treatment phase of the study, and were vaccinated. These 32 patients comprised the study group (Table 2). The majority of patients were Caucasian (72%) and the median age was 26 years (range 20-44). There was equal distribution of CIN 2 and CIN 3 in pretreatment biopsies in the study group.

Adverse Events

All study patients were assessed for adverse events prior to and at various time points up to 41 weeks after vaccination. Adverse effects attributed as at least possibly related to study vaccine are listed in Table 3. Twenty-two of 32 patients (69%) experienced vaccine-specific

related adverse events. The most frequent vaccine-related events were constitutional and local injection site in nature, and were grade 1 or less in severity. Only 55% of patients who received IM vaccination experienced vaccine-specific adverse events, whereas 80% and 73% of patients who received PMED or intralesional vaccination, respectively, experienced vaccine-specific adverse effects. ($p=NS$) No patients had their second or third vaccine held due to adverse events. No grade 3/4 dose-limiting clinical adverse events were reported. No vaccine-related serious adverse events (SAE) were noted. One SAE was reported for a patient who was hospitalized after experiencing bleeding after her LEEP. SAEs were submitted for three patients who became pregnant while on study. Two of these patients experienced miscarriages and one delivered a term infant without complications.

Clinical efficacy

Twenty-seven of 32 patients enrolled on the treatment phase of the trial completed all three vaccinations per protocol and had a standard therapeutic resection performed at week 15. Histologic regression to CIN 1 or less occurred in 8 (30%) patients (Table 4). Persistent CIN 2/3 was observed in 19 (70%) patients. Of the 8 patients with histologic response, 1 had CIN 1 and 7 had no residual CIN. In this pilot study, there was no clear difference in rate of histologic regression across the three different routes of vaccination. No patient had an occult, unsuspected invasive cancer at week 15 in the LEEP specimen.

One patient received all three vaccinations, but became pregnant prior to planned LEEP and was lost to follow up. Four other patients received 1-2 vaccines; one was lost to follow up after vaccination and three had LEEP performed off study. These patients were not included in the analysis of clinical efficacy. Of note, however, no clinical responses were observed in the three patients who had a LEEP off-study.

HPV viral load

Cervical HPV viral loads were measured before and after vaccination in all vaccinated subjects. We found no differences between pre-and post-vaccination viral loads, in any of the treatment cohorts (Figure 1).

Immunogenicity

Peripheral blood T cell responses to the vaccine antigen, HPV16 E7, were measured in all vaccinated patients. Immune responses to E7 were minimal, and were not significantly different than responses to HPV16 E6, which was not included in pNGVL4a-CRT-E7(detox) (Figure 2). However, in the cervical tissue, in subject-matched comparisons, intraepithelial CD8+ T cell infiltrates increased after vaccination in subjects in the intralesional administration cohort ($p = 0.0313$) (Figure 3). The intensity of CD8+ infiltrates in CIN epithelium in subjects who received either intradermal or intramuscular vaccination did not change.

CD8+ T cell infiltrates in the stroma subjacent to residual CIN epithelium increased with all three routes of vaccination. In contrast, in adjacent normal mucosa, the intensity of CD8+ T cell infiltrates did not change, either in the epithelium or in the stroma. The intensity of intraepithelial CD8+ T cell infiltrates in lesional mucosa before vaccination was greater in

both the epithelium as well as the underlying stroma in regressors compared to non-regressors. ($p=0.0477$) (Figure 4).

Discussion

Various therapeutic HPV vaccine strategies have been evaluated in both preclinical and early phase clinical trials. [1,2] These have included live vector, peptide, protein, DNA, RNA replicon, and dendritic cell based HPV vaccines targeting the HPV E6 and E7 oncoproteins. DNA vaccines such as that employed in this study are generally safe, easier to manufacture, and can sustain expression of their constructs longer than RNA vaccines. In general, DNA-based vaccines have shown poor immunogenicity in humans, and require added measures to increase potency. These measures have included enhanced DNA vaccine delivery strategies, such as gene gun, microencapsulation and electroporation, all designed to increase antigen expression in target cells and uptake by dendritic cells. Other potency-enhancing strategies have utilized various adjuvants linked to an oncoprotein-expressing construct in an effort to increase antigen expression, processing, and presentation to dendritic cells.

Several DNA-based therapeutic HPV vaccines have been evaluated in early phase clinical trials in patients with CIN2/3, utilizing various techniques to enhance vaccine potency. [17-23]. These have included 1) ZYC101 (a plasmid encoding an HPV16 E7 HLA-A2-restricted peptide microencapsulated in 1-2 mm PLG microparticles), 2) a next-generation ZYC101 vaccine (a similar vaccine encoding both HPV16 and HPV18 E6 and E7 viral peptides), 3) VGX-3100 (a plasmid vaccine encoding HPV16 and 18 E6 and E7 proteins delivered via intramuscular injection followed by electroporation using a CELLECTRA® constant current device to deliver a small electrical charge); and 4) pNGVL4a-Sig/E7(detox)Sig/Hsp70 (a DNA vaccine that encodes a signal sequence localized to the endoplasmic reticulum (Sig) linked to a mutated HPV16 E7 sequenced and fused to HSP70). The results of these trials have demonstrated in general minimum toxicity (predominantly constitutional and local injection site symptoms) and increased HPV specific immune responses. In spite of these efforts to enhance potency, clinical efficacy remained limited.

In this study, we primarily evaluated the toxicity the novel therapeutic HPV vaccine pNGVL4a-CRT/E7(detox), a plasmid vaccine containing coding sequences for HPV16 E7 linked to calreticulin (CRT), administered via three different routes of vaccination. The potency of this vaccine was enhanced by incorporating calreticulin, a HSP related 46 kDa calcium-binding chaperonin. Calreticulin has been demonstrated to enhance antigen MHC specific presentation and processing and elicit a local anti-angiogenesis effect. Similar to the results of other trials of this nature, the most commonly experienced adverse events were mild constitutional or local injection site symptoms, which were transient and easily medically managed and occurred across the various routes of vaccination.

This trial was also designed to determine in a preliminary manner whether vaccination in the cervical mucosa would elicit greater or more effective immune response compared to peripheral vaccination. The rationale was based on a growing body of literature reporting that antigen-experienced T cells exhibit pronounced tropism for the tissue in which they

encountered their cognate antigen. [24-28] In this study, the intensity of CD8+ infiltrates in cervical dysplastic epithelium increased only in subjects in the intralesional cohort and not in the subjects who received either intramuscular or intradermal vaccination. No subject had significant increases in peripheral blood T cell responses to the HPV16 E7 vaccine antigen. Systemic responses to both E6 and E7 increased slightly at week 19 in all three cohorts, an increase that may have reflected antigen exposure elicited by the LEEP procedure performed at week 15.

This trial was also designed to determine in a preliminary manner whether the anti-tumor response observed in the preclinical model were translated in patients with CIN 2/3. Histologic regression to CIN 1 or less occurred in 9 patients (30%) and was observed in patients in all routes of vaccine administration. While this degree of response was encouraging, it is difficult to state precisely how much pNGVL4a-CRT/E7(detox) vaccination contributed to this rate of regression, as this rate is similar to that observed in unvaccinated patients followed over a similar 15 week timeframe. [29] Of note, HPV viral loads did not decrease significantly, which was also congruent with the histologic endpoints.

Our data underscores the need to evaluate candidate immune therapies in humans. The study vaccine, pNGVL4a-CRT/E7(detox), elicited striking adaptive and innate immune responses to the vaccine antigen in the murine model. [9] In mice, these immune responses had both preventive as well as therapeutic effects against the TC-1 transplantable tumor model. This discrepancy between mice and humans likely reflects critical differences in their biology. For instance, a large component of immunogenicity of naked DNA vaccines is mediated by the unmethylated CpG dinucleotides, which engage TLR9. In mice, TLR9 is expressed by virtually all dendritic cells. In contrast, TLR9 is expressed on plasmacytoid dendritic cells in humans, which are found predominantly in lymphoid tissues and peripheral blood. [30] Nonetheless, some DNA vaccines, when administered with electroporation, have been shown to be immunogenic in humans. [21] These findings denote the importance of the method of DNA delivery and efficiency of *in vivo* transfection in the human context.

The primary limitations of this study include the fact that this was a small phase I trial designed to primarily evaluate the feasibility and safety of pNGVL4a-CRT/E7(detox). Expanded studies will be required to further confirm the potential immunologic benefit of intralesional vaccination over other routes of administration. In addition, additional phase II trials evaluating pNGVL4a-CRT/E7(detox) vaccination at the highest dose level will be required to further assess the clinical benefit of this specific vaccine and whether the potential immunologic benefit of intralesional vaccination translates into better clinical responses.

We are currently investigating whether topical imiquimod will enhance the immune response and therapeutic effect of intralesional pNGVL4a-CRT/E7(detox). Imiquimod is a TLR agonist that is capable of generating robust immune responses and increased potency when used in conjunction with therapeutic HPV vaccines. [31] In preclinical *in vivo* studies, C57BL/6 mice expressing TC1 tumors treated with CRT/E7 DNA and imiquimod were found to have an increased frequency of E7-specific CD8+ T cells within their spleens as compared to the frequency found within the spleens of control mice vaccinated with

CRT/E7 DNA only. Moreover, mice receiving combined therapy experienced tumor reduction and prolonged survival when compared to mice receiving CRT/ E7 DNA vaccination only. Vaccination with the L2E7E6 protein vaccine TA-CIN has also been evaluated in a phase 2 clinical trial after imiquimod application for patients with VIN. [32]

In summary, this trial demonstrated the feasibility, safety and potential clinical benefit of pNGVL4a-CRT/E7(detox) a novel DNA vaccine composed of a pNGVL4a plasmid vector containing a mutated form of the HPV16 E7 antigen linked to calreticulin (CRT) for patients with HPV16 associated CIN2/3. Though a local CD8+ T cell response appeared to be most robust with intralesional vaccination, none of the routes of vaccination were immunogenic to the extent seen in mice and the clinical effect noted was modest. To achieve further success with DNA vaccines of this nature, investigators will need to incorporate further innovations into these vaccines, enhance delivery strategies, identify more useful links between immune response and the desired clinical effect, utilize innovative combinatorial clinical trial designs, and improve upon currently available murine models.

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Research highlights

- The therapeutic HPV DNA vaccine pNGVL4a-CRT/E7(detox) can be administered safely.
- The Immune response was most robust when vaccinated directly into the cervix.
- Histologic regression to CIN 1 was noted in 30% of vaccinated CIN 2/3 patients.

Figure 1. HPV viral load before and after vaccination

HPV copy number equivalents per 1000 cells was determined in exfoliated cell specimens obtained before vaccination (wk 0) and after, at the time of therapeutic resection (wk 15), in subjects who received pNGVL4a-CRT/E7(detox) either intradermally (9), intramucosally (9), or intramuscularly (9).

Figure 2. Peripheral blood T cell responses to HPV16 E7 and E6

Cellular immune responses in unfractionated PBMC, quantified by IFN- γ ELISpot assays.

Subjects received a total of three doses of either 8 (3) or 16 (6) μ g of pNGVL4a-CRT/E7(detox) administered intradermally; either 1 (3) or 3 (6) mg of vaccine administered intramucosally; or either 1 (3) or 3 (6) mg of vaccine administered intramuscularly in the deltoid. Results are expressed as spot-forming units (SFUs) per 10⁶ peripheral blood mononuclear cells (PBMCs). Responses to vaccine antigen, E7, are depicted in the left panel, and responses to E6, which was not a vaccine antigen, are depicted in the right panel. Subjects with histologic regression at week 15 are depicted in red.

Figure 3. Tissue CD8+ infiltrates in the target tissue before and after vaccination
Representative IHC staining for CD8 in lesional tissue before (left column) and after (right column) vaccination, in subjects vaccinated (A) intradermally, (B) intramucosally, or (C) intramuscularly. (D) Bar graphs depicting quantitated CD8+ infiltrates in the epithelium and stroma of CIN and normal mucosa, before and after vaccination, in all treatment cohorts. Data in bar graphs are means of 3-10 regions of interest quantitated per tissue compartment, per subject. * $p < 0.05$, Wilcoxon signed rank test. "e" epithelium, "s" stroma, filled in black dot, epithelium, circle, stroma; regressors are depicted in red. Bars 100 μm .

Figure 4. Intensity of mucosal CD8+ T cell infiltrates before vaccination correlates with subsequent histologic regression

Quantification of CD8+ infiltrates in lesional epithelium before vaccination, at study entry, in lesional epithelium and stroma quantitated separately (left panel) and in toto (right panel). At least 3 and up to 10 fields from each tissue section were quantitated.

Table 1**Treatment Cohorts**

Cohort	Route of Delivery	Dose
1	PMED	8 µg
2	PMED	16 µg
3	Intramuscular	1 mg
4	Intramuscular	3 mg
5	Intralesional	1 mg
6	Intralesional	3 mg

Table 2
Patient Characteristics

Characteristic	PMED (n=10)	Intramuscular (n=11)	Intralesional (n=11)	Total (n=32)
Age				
Median	25.5	26	26	26
(Range)	(20,44)	(21,35)	(22,35)	(20,44)
Race n (%)				
Caucasian	9(90)	5(45)	9(82)	23(72)
AA	1(10)	5(45)	2(18)	8(25)
Asian	0(0)	1(10)	0(0)	1(3)
BMI				
Mean (SD)	27.8(6.6)	28.6 (6.9)	28.1(8.3)	28.2(7.1)
Median (Range)	29.6 (18.4, 38.8)	26.3 (21.8, 46.7)	27.4 (18.7, 42.3)	27.53 (18.4,46.7)
Smoker n (%)				
Yes	8 (80)	3(27)	10(91)	21(65.63)
No	2 (20)	8(73)	1(9)	11(34.38)
Dysplasia n (%)				
CIN 2	5 (50)	6(55)	4(36)	15(47)
CIN 3	4 (40)	4(36)	7(64)	15(47)
CIN 2/3	1 (10)	1(9)	0	2(6)

BMI: Body Mass Index; SD: Standard Deviation

Table 3

Vaccine Specific Adverse Events¹

Adverse Event	TOTAL (n=22/32)		PMED (n=8/10)		Intramuscular (n=6/11)		Intravesical (n=8/11)	
	Grade 0	1	Grade 0	1	Grade 0	1	Grade 0	1
Total Occurrences	188	2	92	2	51	0	45	0
Local site injection								
	54	0	43	0	10	0	1	0
Bruise	1	0	0	0	1	0	0	0
Discoloration	19	0	19	0	0	0	0	0
Numbness	1	0	1	0	0	0	0	0
Pain	19	0	12	0	6	0	1	0
Reaction	14	0	11	0	3	0	0	0
General Pain, Fever, Malaise/Fatigue								
	121	2	42	2	38	0	41	0
Abdominal Discomfort	9	0	0	0	1	0	8	0
Cramping	8	0	0	0	0	0	8	0
Fatigue	35	0	13	0	14	0	8	0
Flu like reaction	7	0	3	0	3	0	1	0
Headache	33	1	13	1	8	0	12	0
Muscle/Joint Aches	20	0	8	0	8	0	4	0
Nausea	3	0	0	0	3	0	0	0
Pain	1	1	0	1	1	0	0	0
Pruritis	5	0	5	0	0	0	0	0
Other								
	12	0	7	0	5	0	3	0
Depressed	2	0	2	0	0	0	0	0
Limb Numbness	5	0	5	0	1	0	0	0
Rash	2	0	0	0	2	0	0	0
Spotting	2	0	0	0	0	0	2	0
Vaginal Bleeding	1	0	0	0	0	0	1	0

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Adverse Event	TOTAL (n=22/32)		PMED (n=8/10)		Intramuscular (n=6/11)		Intravesical (n=8/11)	
	Grade 0	1	Grade 0	1	Grade 0	1	Grade 0	1
Laryngeal inflammation			0	0	2	0	0	0

Adverse event relation to study drug is possible, probably, or definite

Table 4
Clinical Efficacy

	PMED		Intramuscular		Intralesional		Total
	8 mcg	16 mcg	1 mg	3 mg	1 mg	3 mg	
Total patients	3	6	3	6	3	6	27
Best Response							
None ¹	3	4	2	4	1	5	19(70%)
Partial ²	0	0	1	0	0	0	8(30%)
Complete ³	0	2	0	2	2	1	

¹Residual CIN 2/3

²Residual CIN 1

³No residual CIN