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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)

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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.

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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 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.

1. 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

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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.

2. Methods

2.1. 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).

2.2. 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).

2.3. 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 weeks 8–10 (t2), at study week 15 (t3), and at study week 19 (t4).

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