A Phase I Efficacy and Safety Study of HPV16-Specific Therapeutic DNA-rVaccinia Vaccination in Combination with Topical Imiquimod in patients with HPV16+ High Grade Cervical Dysplasia (CIN3)

Scientific Abstract

Background
In the United States, despite the availability of inexpensive, noninvasive screening, cervical cancer remains the sixth most commonly diagnosed malignancy among women. Over the past decade, SEER data have demonstrated a 17% increase in incidence in the U.S., normalized for population growth, with a disproportionate increase among young women. Current primary therapies include radical surgery and chemoradiation. For those with recurrent disease, a combination of further surgery including total pelvic exenteration, radiation, and chemotherapy may be used. However these modalities are associated with significant treatment toxicity, and overall survival remains a dismal 40%. One of the best strategies to decrease the disease burden of cervical cancer is to intervene in patients with premalignant disease of the cervix. The identification of the causal association of high risk strains of human papillomavirus (HPV) with premalignant disease of the cervix provides an ideal opportunity to develop vaccines targeted at HPV+ premalignant lesions. HPV16 is associated with over half of all cervical cancers and precursor lesions (CIN2/3).

We have developed a therapeutic DNA vaccine which targets the HPV16 E7 oncoprotein, which is consistently expressed in cancerous and precancerous epithelial cells, but not in normal tissue. Phase I evaluation of this vaccine, pNGVL4a-Sig/E7(detox)/HSP70, has demonstrated feasibility, immunogenicity, and absence of toxicity in this patient population.

Nevertheless, because DNA vaccination has been shown to have relatively limited potency in humans, we have explored the use of a heterologous prime-boost vaccination strategy to enhance immunogenicity. Preclinical evaluation of a regimen that tested a DNA-E7 vaccine to prime an antigen specific T cell response, followed by boost vaccination with a recombinant vaccinia-E7, showed dramatical immune responses and antitumor effects against E7 expressing epithelial tumors. Similar DNA-vaccinia prime-boost regimens, targeted at malarial and HIV antigens, have demonstrated safety, tolerability, and significantly enhanced immune responses in healthy volunteers.

Hence we propose evaluating a heterologous prime-boost vaccination strategy against the HPV16 E7 oncoprotein in a phase I clinical trial in healthy women with HPV16+ CIN3. The regimen will consist of sequential immunization with two HPV16 E7- expressing vaccine constructs, the pNGVL4a-Sig/E7(detox)/HSP70 DNA vaccine, as the priming vaccination, followed by boost immunization with the recombinant, Wyeth strain derived, vaccinia construct, TA-HPV. The safety, tolerability, and immunogenicity of both vaccine constructs in this patient population, have been assessed in completed Phase I studies.

Objectives
1) To characterize safety and tolerability associated with this vaccination regimen in
otherwise healthy, HPV16+ CIN3 patients.

2) To evaluate the effect(s) on the CIN histology after priming vaccination with pNGVL4a-Sig/E7(detox)/HSP70 and booster immunization with TA-HPV. The evaluation will be based on the regression of CIN3 at week 28, assessed as no CIN3 lesion detected by colposcopy/biopsy and Pap smear.

3) To explore the effects of immunotherapy on cervical HPV viral load.

4) To demonstrate specific in-vivo parameters of immune response as a consequence of the vaccination regimen.

5) To correlate in-vivo measures of immune response with clinical response.

6) To investigate whether vaccine efficacy can be improved by cervical application of imiquimod.

Patient Population
A maximum of thirty six healthy women with colposcopically-directed, biopsy-confirmed CIN3, caused by HPV16.

Study Design
Phase I, open label efficacy and safety study trial. Treatment will consist of 2 sequential plasmid DNA vaccinations, followed by a recombinant vaccinia boost. Patients will be enrolled into 1 of 5 treatment groups, which will be filled sequentially. The first treatment group will undergo DNA priming followed by low dose TA-HPV boost (1.6x10^5 pfu) to assess the safety and tolerability of this regimen. The second group will receive an intermediate dose of TA-HPV boost (1.6x10^6 pfu). The third group will employ a full dose TA-HPV boost (1.6x10^7 pfu), that is comparable to the dosing used in other human studies. The fourth treatment group will be an imiquimod-alone run-in group to assess the tolerability of this therapy. The fifth and last treatment group will integrate 2 DNA vaccination, the full dose TA-HPV boost (1.6x10^5 pfu) and topical imiquimod at the time of each vaccination.

Treatment Plan and Schedule
Patients will first undergo a routine diagnostic workup for CIN3, before receiving treatment. Treatment will consist of two doses of the pNGVL4a-Sig/E7(detox)/HSP70 DNA vaccine at 4-week intervals, followed by a TA-HPV boost four weeks after the last DNA vaccine. The first 3 patients will be a run-in treatment group who will be treated with plasmid DNA vaccination and a low dose TA-HPV boost. A second group of 3 patients will follow and be treated with plasmid DNA vaccination and an intermediate dose TA-HPV boost. The third group consisting of 12 patients will be treated with plasmid DNA vaccination, followed by a full dose of TA-HPV boost. The next 6 patients in the fourth group will be treated only with 3 doses of imiquimod alone on the site of the lesion, administered at 4 week intervals. After the imiquimod alone run-in is completed, the 12 remaining patients in the fifth group will be treated with imiquimod on the site of the lesion at the time of each vaccination.
### Summary of the Study Design

<table>
<thead>
<tr>
<th>Group</th>
<th>DNA Vaccine Dose</th>
<th>TA-HPV Dose</th>
<th>Topical Imiquimod</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 mg</td>
<td>$1.6 \times 10^5$ pfu</td>
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<td>3</td>
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<td>$1.6 \times 10^6$ pfu</td>
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<td>3</td>
</tr>
<tr>
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<td>3 mg</td>
<td>$1.6 \times 10^7$ pfu</td>
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<td>12</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>None</td>
<td>3 times total, at 4-week intervals</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>3 mg</td>
<td>$1.6 \times 10^7$ pfu</td>
<td>3 times total, at 4-week intervals, at the time of vaccination</td>
<td>12</td>
</tr>
</tbody>
</table>

#### Dose

Both the pNGVL4a-Sig/E7(detox)/HSP 70 DNA and the TA-HPV vaccines will be administered as intramuscular injections in the deltoid muscle. The pNGVL4a-Sig/E7(detox)/HSP70 DNA will be administered at the 3 mg dose level, while three dose levels of TA-HPV inoculations will be explored. The first treatment group will be dosed at $1.6 \times 10^5$ pfu to assess the safety of this proposed regimen before proceeding to the next dose of $1.6 \times 10^6$ and then to the full dose of $1.6 \times 10^7$ pfu. Imiquimod cream 5% will be applied directly to the cervical lesions in patients enrolled in Groups 4 and 5.

Accrual to the study will continue until every treatment position is evaluated, or dose-limiting toxicities are observed. No dose-limiting toxicities have been observed at any dose level of DNA vaccination in the literature.

#### Dose-limiting toxicity

Based on previous clinical experience with both vaccine vectors used alone as well as in similar prime-boost vaccination regimens, no dose-limiting or severe toxicities are expected.

#### Safety Evaluation

The toxicity data will be descriptive, characterized according to the NCI CTC v3.0. Monitoring for adverse events will occur internally on real time, at regular, weekly meetings of the Johns Hopkins Oncology Center Clinical Trials Working Group, and annually, by the Hopkins Oncology Center Clinical Research Review & Monitoring Committee.

#### Product

The vaccine, pNGVL4a-Sig/E7(detox)/HSP70, is a DNA vaccine targeted at the Human Papillomavirus (HPV) 16 E7 antigen, for the treatment of patients with high grade, pre-invasive HPV16 intraepithelial lesions (HGSILs) of the cervix. The vaccine is composed of a naked
plasmid DNA vector, pNGVL4a, into which the antigen of interest, HPV16 E7(detox), linked to targeting sequences which enhance the potency of intramuscular (IM) DNA vaccination, namely, signal peptide (Sig), and mycobacterium tuberculosis heat shock protein 70 (HSP70), has been cloned. Clinical grade drug has been manufactured via the NCI RAID mechanism.

TA-HPV is a genetically engineered therapeutic product, which has been developed as a potential treatment for late stages of CIN, invasive cervical cancer and other HPV related neoplasias of the female genital tract. Specifically, TA-HPV is a recombinant vaccinia virus, v9a.1, derived from the Wyeth (New York Board of Health) strain, and engineered to express modified E6 and E7 genes from HPV types 16 and 18. Clinical grade vaccine was provided by Celtic Pharma.