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# A phase I study to evaluate a human papillomavirus (HPV) type 18 L1 VLP vaccine☆

Kevin A. Ault[a,*], Anna R. Giuliano[b], Robert P. Edwards[c,1], Gretchen Tamms[d], Lee-Lian Kim[d], Judith F. Smith[d], Kathrin U. Jansen[d], Maria Allende[d], Frank J. Taddeo[d], DeeMarie Skulsky[d], Eliav Barr[d]

[a] *Department of Obstetrics and Gynecology, University of Iowa Hospitals and Clinics, 200 Hawkins Drive, Iowa City, IA 52242, USA*

[b] *Arizona Cancer Center, University of Arizona, Tucson, AZ, USA*

[c] *Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA*

[d] *Merck Research Laboratories, Merck and Co. Inc., West Point, PA, USA*

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**Abstract**

Human papillomavirus (HPV) infection can cause genital warts and cervical cancer. HPV types 6 and 11 cause >90% of genital wart cases; HPV16 and 18 cause 70% of cervical cancers. A prophylactic HPV (types 6, 11, 16, 18) L1 virus-like particle (VLP) vaccine may substantially reduce the incidence of these lesions. This report describes the results of a phase I study of the HPV18 component of such a vaccine. Forty women were randomized to receive either HPV18 L1 VLP vaccine or placebo. Anti-HPV18 responses were measured using a competitive radioimmunoassay (cRIA). Tolerability was evaluated using vaccination report cards (VRC). The study showed that the HPV18 L1 VLP vaccine was generally well-tolerated and highly immunogenic. Peak anti-HPV18 geometric mean titers (GMT) in vaccines were 60-fold greater than those observed in women following natural HPV18 infection. Further studies of a multivalent HPV L1 VLP vaccines are warranted.



*Keywords:* Human papilloma virus; Vaccination; Serology

## 1. Introduction

Human papilloma virus (HPV) infects >50% of sexually-active adults [1] and is responsible for genital warts and cervical intraepithelial neoplasia (CIN), an abnormality that may lead to cancer. Four HPV types have been associated with the majority of these diseases: HPV6 and 11 cause >90% of genital wart cases, HPV16 causes 50% of cervical cancer cases, and HPV18 causes 20% of cervical cancer cases. HPV18-related cervical cancers are associated with especially high mortality, because they often present as adenocarcinomas [2], lesions that are poorly detected by Pap testing and are difficult to treat [3].

Vaccination against common HPV types may reduce the burden of HPV-related diseases. HPV L1 capsid proteins produced in modified yeast self-assemble into empty virus-like particles (VLPs) that resemble the wild-type virion but are non-infectious. In several early phase clinical studies, HPV11 and 16 L1 VLP vaccines were observed to be generally well-tolerated and to induce substantial anti-HPV11 and 16 responses, respectively [4–6]. In two recent studies, administration of an HPV16 L1 VLP vaccine was shown to prevent HPV16 infection and related CIN in 100% of HPV16-naïve vaccines [5,6].

Because administration of HPV L1 VLP vaccine is likely to result in type-specific immunity, prophylactic vaccines that target the majority of cancer-causing HPV types should include both HPV16 and HPV18 components. Here we describe a phase I clinical trial that investigated the immunogenicity and tolerability of the HPV18 L1 VLP component of such a vaccine.

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* Corresponding author. Tel.: +1-319-353-7384; fax: +1-319-356-3901.
*E-mail address:* kevin-ault@uiowa.edu (K.A. Ault).

[1] Present address. Department of Obstetrics and Gynecology, University of Louisville, AY 300550 South Jackson Street, Louisville, KY 40202, USA.

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## 2. Methods

A double-blind, randomized, placebo-controlled phase I study of an HPV18 L1 VLP vaccine (Merck Research Laboratories, West Point, PA) was conducted in 40, 16–23-year-old women recruited from three US college campuses. Potential participants were excluded if they reported a prior abnormal Pap smear or more than five lifetime male sexual partners. Additionally, women were not required to be HPV18-seronegative or PCR negative upon enrolment.

Volunteers were randomized in a 2:1 vaccine to placebo ratio. Each dose of HPV18 L1 VLP vaccine consisted of 80 μg of HPV18 L1 VLP formulated with 450 μg of amorphous aluminum hydroxyphosphate sulfate adjuvant in a buffered solution yielding a total injection volume of 0.5 ml. The 80 μg dose, the highest dose contemplated for Merck's investigational quadrivalent HPV (types 6, 11, 16, 18) L1 VLP vaccine, was selected because the current study was intended to study the vaccine's tolerability. Placebo vaccination consisted of the aluminum adjuvant and buffered solution only. Participants were vaccinated at day 0, months 2, and 6. The vaccine and placebo were visually indistinguishable. Participants were required to use effective contraception during the trial.

At day 0 and month 7, participants underwent physical examination, genital sampling for HPV, and Pap testing (ThinPrep$^{TM}$, Cytyc, Boxborough, MA).

Swabs collected from the cervix, vagina, labia, vulva, perineum, and perianal area were tested for the presence of HPV18 DNA using a polymerase chain reaction (PCR) assay targeting the HPV18 L1, E6 and E7 genes as previously described [5]. Any sample testing positive for two or three of these genes was considered positive. Serum samples were collected at day 0 and months 2, 3, 6, and 7 to measure anti-HPV18 responses using a competitive radioimmunoassay (cRIA). This assay is similar to a previously published anti-HPV16 VLP cRIA [5]. These assays are based on the competition of a HPV type-specific, neutralizing monoclonal antibody with test sera for a limited amount of binding sites presented by HPV L1 VLPs coated onto beads. Bound monoclonal antibody is detected with $^{125}$I-anti-mAb. Antibody titers were determined relative to a non-human primate reference serum generated by immunization with HPV18 L1 VLP and were expressed in arbitrary units (milliMerck units/ml, mMU/ml). An acceptable anti-HPV18 response was defined as an anti-HPV18 level $\geq$200 mMU/ml 1-month after completion of the 3-dose vaccination regimen. In one clinical study with a prototype HPV11 VLP vaccine, anti-HPV levels have been shown to correlate with the capacity to neutralize live HPV virions and with long-term seropositivity [7].

To measure tolerability, participants completed vaccination report cards (VRC) after each vaccination. The VRCs asked participants to report injection site or systemic complaints that occurred during the 14 days following vaccination. The VRC also required participants to record their oral temperature 4 h after vaccination, and daily for each of the ensuing 4 days. Adverse experiences (AEs) were defined as new physical abnormalities or worsening of pre-existing abnormalities that occurred during the 14 days following vaccination. Participants were asked to categorize the intensity of each adverse experience using pre-specified criteria.

An immune response resulting from an ongoing or antecedent HPV18 infection may magnify vaccine-induced anti-HPV18 responses. Thus, the primary evaluation of immunogenicity was performed in women who were HPV18-naïve at enrollment (HPV18 PCR- and seronegative on day 0) and who had not acquired HPV18 infection during the study (HPV18 PCR negative at month 7). The protocol pre-specified that the primary analysis would be conducted in women who had received all 3 doses of study vaccine, had undergone serologic testing at month 7, and had not received immunosuppressive medications or blood products during the course of the study.

The primary immunogenicity analysis was performed at month 7. Geometric mean titers (GMTs) and the percentage of participants who achieved an anti-HPV18 level $\geq$200 mMU/ml were computed. Since the goal of the study was to evaluate whether or not the vaccine would induce anti-HPV18 levels $\geq$200 mMU/ml in greater than half of the vaccinated subjects, the percentage of subjects with anti-HPV 18 $\geq$ 200 mMU/ml was computed separately among HPV18 L1 VLP vaccine and placebo recipients and compared to a reference of 50% using exact binomial testing for a single proportion. Two-sided $P$ values less than 0.05 were considered statistically significant. Immunogenicity also was evaluated postdose 2 (month 3).

## 3. Results

Key demographic characteristics were generally comparable between treatment groups. The mean age of participants was 20.7 years. Few participants reported prior gynecologic infections: two participants in the placebo group had previous occurrences of genital warts, one in the placebo group had bacterial vaginosis, and two (one each in the vaccine and placebo groups) had Chlamydia infection. The median number of lifetime male sexual partners was 2.0.

A total of 22 women in the vaccine group and 11 women in the placebo group were included in the per-protocol immunogenicity evaluation. Of the seven women who were excluded, three failed to complete the protocol (one was lost to follow-up, one withdrew consent, and one experienced hives and was withdrawn from the study), two were HPV18 PCR positive at enrollment and at month 7, one received a blood transfusion for gastrointestinal bleeding, and one was excluded because her month 7 serum sample was not obtained within the pre-specified time period following the third vaccination.

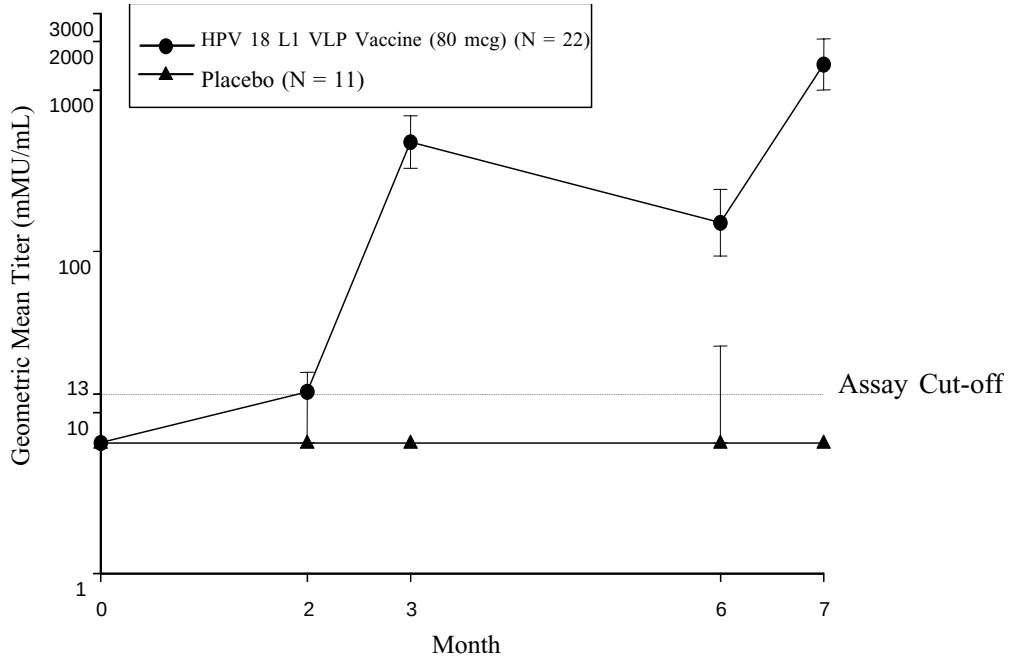


Fig. 1. Serological responses to HPV18 L1 VLP expressed as anti-HPV18 cRIA GMTs in mMU/ml on a logarithmic scale with 95% tile confidence intervals. A GMT of 6.5 mMU/ml indicates a titer below the assay cutoff of 13 mMU/ml.

In the per-protocol cohort, all of the women in the vaccine group and none in the placebo group developed anti-HPV18 responses 1-month postdose 2 and 3. Serological responses are summarized in Fig. 1. Responders are women who seroconverted (above assay cutoff of 13 mMU/ml) following the administration of either the HPV18 L1 VLP vaccine or placebo. Of the vaccine recipients, 86 and 100% achieved anti-HPV18 levels $\geq$200 mMU/ml at months 3 and 7, respectively. The proportion of subjects with anti-HPV 18 $\geq$ 200 mMU/ml was significantly greater than 50% ($P < 0.001$) at both months 3 and 7. Anti-HPV18 responses increased after each dose of vaccine.

Adverse experiences 0–14 days following any dose were common among both vaccine and placebo recipients (Tables 1 and 2). All enrolled women were evaluated for AEs. Erythema at the injection site appeared more frequently in the vaccine group, (40.7%), compared with the placebo group, (7.7%), and the difference was nominally statistically significant ($P = 0.035$, unadjusted for multiple comparisons). No other statistically significant differences between placebo and vaccine groups were observed. None of the injection site AEs were judged by the participants to be severe in intensity. The most common injection site AE was pain. Most of the systemic AEs were judged by the participants to be mild or moderate in severity. (Table 2) The proportion of systemic AEs judged to be severe in intensity were 6.6 and 15.7% in the vaccine and placebo groups, respectively. Systemic AEs are summarized in Table 2. Only systemic AEs that affected more than 5% of women receiving vaccine are shown. Headache was the most commonly reported systemic adverse experience. Systemic AEs were not different between women receiving vaccine and those receiving placebo.

Table 1
Local adverse events

| | Vaccine ($N = 27$) | | Placebo ($N = 13$) | |
|---|---|---|---|---|
| | *N* | (%) | *N* | (%) |
| Number (%) of subjects with one or more adverse site reactions | 26 | 96 | 11 | 85 |
| Ecchymosis | 1 | 4 | 0 | 0 |
| Erythema | 11 | 41 | 1 | 8 |
| Pain, tenderness and/or soreness | 26 | 96 | 11 | 85 |
| Pruritis | 2 | 7 | 0 | 0 |
| Swelling | 10 | 37 | 3 | 23 |

Table 2
Systemic adverse events

| | Vaccine ($N = 27$) | | Placebo ($N = 13$) | |
|---|---|---|---|---|
| | *N* | (%) | *N* | (%) |
| Number (%) of subjects with one or more systemic adverse experience | 19 | 70 | 11 | 85 |
| Fatigue | 2 | 7 | 2 | 15 |
| Abdominal pain | 2 | 7 | 1 | 8 |
| Diarrhea | 3 | 11 | 1 | 8 |
| Nausea | 2 | 7 | 0 | 0 |
| Headache | 13 | 48 | 8 | 62 |
| Upper respiratory infection | 2 | 7 | 2 | 15 |
| Pharyngitis | 2 | 7 | 4 | 31 |
| Sinus disorder | 2 | 7 | 0 | 0 |
| Menstrual disorder | 2 | 7 | 0 | 0 |

## 4. Discussion

In this study, administration of a 3-dose regimen of HPV18 L1 VLP vaccine was generally well-tolerated and induced high titer anti-HPV18 levels in all participants.

In previous studies, administration of HPV16 and HPV11 L1 VLP vaccines induced substantial peak serum anti-HPV levels. Brown et al. [7] further demonstrated that vaccine-induced anti-HPV11 antibodies neutralized a large input load of live HPV11 virions. Evan et al. [4] and Harro et al. [6] have reported phase I trials of HPV16 VLP vaccines. Their findings concerning immunogencity and tolerability are similar to this report. Finally, two sets of studies demonstrated that administration of HPV16 L1 VLP vaccine protected HPV16-naïve women from acquisition of HPV16 infection [5]. These data suggest that a vaccine containing HPV18 L1 VLPs may protect against HPV18 infection and related disease. Furthermore, although the HPV L1 VLP dose used in this study was twice the dose of VLP 16 L1 used in a previous clinical trial of VLP L1 vaccine [5], the safety profiles were similar.

Although these preliminary studies of HPV L1 VLP vaccines are encouraging, two questions affecting their ultimate utility remain. First, the minimum protective anti-HPV level is unknown. In this report, peak vaccine-induced anti-HPV18 responses were 60-fold higher than anti-HPV18 responses observed in women with detectable serum anti-HPV18 following clearance of HPV18 infection. The duration of these antibody responses remains to be determined.

Second, the impact of prophylactic HPV vaccines on cervical cancer rates remain to be determined. Most HPV infections and low grade CIN clear without sequelae. CIN 2/3 lesions, on the other hand, are the immediate and obligate precursors to cervical cancer. Conservative regimens of screening and excision of CIN 2/3 lesions have resulted in large reductions in cervical cancer rates [8,9]. Thus, for use in cervical cancer prevention, vaccines targeting HPV18 or other cancer-causing HPV types must demonstrate reduction in the incidence of CIN 2/3 lesions related to these types.

The ideal prophylactic HPV L1 VLP vaccine should maximize coverage of pathogenic HPV types. A vaccine targeting HPV 6, 11, 16, and 18 has the potential to impact over 90% of genital wart cases, 50% of CIN 1 cases, and 70% of CIN 2/3 and cervical cancer cases. Studies of such a vaccine are underway. Further evidence supporting a multivalent vaccine has recently been published by Munoz et al. [10]. A vaccine containing the eight most common oncogenic HPV types could potentially prevent 95% of cervical cancers. Thus, an effective and well-tolerated vaccine aimed at these common HPV types may substantially reduce the clinical and socioeconomic costs of HPV infection.

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