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Early assessment of the efficacy of a human papillomavirus type 16 L1 virus-like particle vaccine

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Abstract

A post hoc analysis was performed using combined data from two Phase I tolerability/immunogenicity studies of monovalent human papillomavirus type 11 (HPV11) or HPV16 L1 virus-like particle (VLP) vaccines. The goal was to determine if the HPV16 L1 VLP vaccine protected against HPV16 infection. Vaccine or placebo was given at 0, 2 and 6 months. HPV16 infection was defined by positive polymerase chain reaction (PCR) results following vaccination. The incidence of HPV infection was observed to be 0 cases per 100 person-years at risk in the vaccine group, and 5 cases per 100 person-years at risk in the control group. These results support the institution of larger efficacy trials for HPV L1 VLP vaccines.
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1. Introduction

Human papillomavirus (HPV) infection is the most common sexually transmitted disease. Approximately, 35 HPV types are known to infect human genitalia, causing a range of clinical states including asymptomatic infection, genital warts, cytologic abnormalities of the cervix, and invasive cervical cancer. HPV16 is an oncogenic type, detected in 25% of low-grade cervical intraepithelial neoplasias, 50% of high-grade cervical intraepithelial neoplasias, and approximately 50% of cervical cancers [1–3]. Most HPV infections become undetectable over time and do not lead to cervical dysplasia. Nevertheless, a subset of women who have persistent infection with an oncogenic HPV type is at risk for development of cervical cancer [4–6]. A prophylactic vaccine that prevents HPV16 infection could therefore substantially reduce the burden of HPV-related cervical disease.

The immune responses that protect against HPV16 infection and disease have not been completely defined. The immunogenicity of HPV involves presentation to the immune system of conformational epitopes displayed on viral capsids. The L1 major capsid protein can be expressed in yeast or Sf9 insect cells, and self assembles into virus-like particles, or VLPs [7–11]. Recombinant L1 VLPs appear identical to infectious virions in electron micrographs and contain conformation-dependent neutralizing epitopes [12]. Preclinical studies indicate that vaccination with L1 VLPs induces neutralizing antibodies and provides protection from virus challenge [13–17].

Studies of HPV16 L1 VLP vaccines have shown that they are well tolerated and generate high levels of anti-HPV16 [18,19]. Recently, the primary analysis of the first controlled trial of an HPV16 L1 VLP vaccine was reported [19]. In that study, 2392 women (16–23 years old) were randomized to receive placebo or three doses of 40 μg of HPV16 VLP vaccine in a 0, 2 and 6 month regimen. Genital samples for HPV DNA were obtained at enrollment and every 6 months. Biopsy tissue from participants with abnormal Papanicolaou smears was evaluated for cervical intraepithelial neoplasia and analyzed by polymerase chain reaction (PCR) for HPV16 DNA. The median duration of follow-up was 17.4 months following completion of the vaccination regimen. The incidence of persistent HPV16

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infection was 3.8/100 participant-years at risk in the placebo group and 0.0/100 participant-years at risk in the vaccine group (100% efficacy, 95% confidence interval, 90–100%; $P < 0.0001$). All nine cases of HPV16-related cervical intraepithelial neoplasia occurred among placebo recipients. It was concluded that administration of the HPV16 L1 VLP vaccine reduced the incidence of HPV16 infections and HPV16-related cervical intraepithelial neoplasia.

The goal of the current post hoc analysis was to provide further evidence for efficacy prior to initiation of large-scale studies of L1 VLP vaccines. We therefore conducted a combined analysis of two randomized, placebo-controlled Phase I clinical studies of HPV11 and HPV16 L1 VLP vaccines. Although the main objectives of these similarly designed studies were to evaluate the vaccines' tolerability and immunogenicity, study participants also underwent cervicovaginal sampling for HPV DNA detection at regular intervals. Thus, these two studies were suitable to evaluate whether administration of HPV16 L1 VLP vaccine could reduce the incidence of HPV16 infection in participants who were HPV16-naïve at enrollment. Since HPV16 infection is an obligate step to the development of HPV16-related cervical cancer, such a demonstration would provide a preliminary indication of the potential protective efficacy of such a vaccine with respect to well-defined clinical endpoints.

2. Materials and methods

2.1. Study participants and vaccination

Local Institutional Review Board approval was obtained prior to study initiation, and all study participants provided informed consent. Protocols 001 and 002 were placebo-controlled dose-ranging studies of HPV11 or 16 L1 VLP vaccines, respectively, produced in yeast. Protocol 001 randomized 140 women in a 1:1:1:1:1 ratio to receive intramuscular injections of one of four dose formulations of HPV11 L1 VLP vaccine (10, 20, 50, or 100 μg with 225 μg of aluminum adjuvant) or placebo (containing only aluminum adjuvant) at Months 0, 2 and 6. Approximately 50% of Protocol 001 participants in each of the 20, 50, and 100 μg dose groups also received a fourth dose of HPV11 vaccine or placebo at Month 12. Protocol 002 randomized 109 women to receive intramuscular injections of one of three dose formulations of HPV16 L1 VLP vaccine (10, 40, or 80 μg with aluminum adjuvant) or placebo (containing only aluminum adjuvant) at Months 0, 2 and 6. Originally, Protocol 002 was designed such that women would be randomized in a 1:1:1:1 ratio to the four treatment groups. Nevertheless, early in Protocol 002, the 10 μg HPV16 L1 VLP vaccine formulation failed to meet stability specifications. The use of this formulation was discontinued, and study participants allocated to the 10 μg dose group received 40 μg for all subsequent injections. Thus, the number of participants in the 40 μg dose formulation in Protocol 002 was larger than originally intended and this provided more information on this dose.

To be eligible for enrollment, potential participants were required to be healthy 16–23 year-old-women with a lifetime history of zero to five male sexual partners. The main exclusion criteria included a lifetime history of greater than five male sexual partners and a history of abnormal cervical cytology or genital warts.

2.2. Collection of specimens

Specimens for type-specific HPV PCR were collected at a screening visit (Days 30–14), and again at Day 0, Months 3, 7, 12, 24 and 36. Six specimens were obtained for HPV PCR at the screening visit and at Month 7: a labial/vulvar/perineal swab, cervicovaginal lavage, lateral vaginal swab, endo/ecto cervical swab, perianal/anal swab, and an oral swab. Only the first four specimens listed above were obtained at all the other time points. Serum also was obtained at all time points for evaluation by a type-specific anti-HPV radioimmunoassay. In Protocol 001, participants who were HPV6 or 11 PCR positive or HPV11 seropositive at the screening visit were not enrolled in the study. In Protocol 002, HPV16 PCR positive or seropositive participants were not enrolled in the study.

2.3. PCR and serologic assays

Cervicovaginal lavage and swab samples were prepared for PCR using a Qiaquick kit to extract and purify DNA (Qiagen Inc., Valencia, CA). HPV types 6, 11, 16, and 18 DNA was detected by PCR using HPV type-specific primers based on the HPV L1, E6 and E7 genes (HPV type-specific PCR). Assays were performed in a 96 well format with 50 μl per well volumes. Each well contained 46 μl of master mix and 4 μl of purified sample DNA or appropriate control (known amounts of a specific HPV type, or no HPV DNA). Master mix contained 5 μl 10× PCR Buffer II (Perkin Elmer), 4 μl 25 mM $MgCl_2$, 0.4 μl of 25 mM dNTPs, 0.25 μl of 5U/μl Amplitaq Gold polymerase (Perkin Elmer), 0.25 μl each of 500 μg/ml sense and antisense primers appropriate for the reaction being performed. Primer sequences for human β-globin 5′-GAA GAG CCA AGG ACA GGT AC-3′ and 5′-CAA CTT CAT CCA CGT TCA CC-3′. Primer sequences for the HPV types 6, 11, 16, and 18 PCR assays are indicated in Table 1. As a positive control for PCR, 100 copies of an appropriate plasmid control were added to two separate sample wells on each plate. PCR cycling conditions were determined empirically to yield optimal amplification. PCR products were dot blotted onto positively charged nylon membranes (Hybond N+, Amersham), dried and UV cross-linked (UV Stratalinker, Stratagene Inc.) until ready for hybridization and detection. Type- and gene-specific probes were labeled with ^{32}P-ATP using T4 polynucleotide kinase. The oligonucleotide DNA probe sequence for the human β-globin gene

Table 1
Primer and probe sequences for HPV6, 11, 16 and 18 PCR/hybridization assays

	E6	E7	L1
HPV 6	Forward: 5′-ATG GAA AGT GCA AAT GCC TCC-3′ Reverse: 5′-GGG TAA CAT GTC TTC CAT GCA-3′ Probe: 5′-AAA CAA GAC ATC TTA GAC GTG-3′	Forward: 5′-ATG TTA CCC TAA AGG ATA TTG TAT-3′ Reverse: 5′-CAC TAT GTC TAG TGT TCC CAA-3′ Probe: 5′-GTG GAC GGA CAA GAT TCA CAA C-3′	Forward: 5′-AAT TCA GGG AGT GGT GGT AAC-3 Reverse: 5′-GAC GTG CGA TTT CCA CTA CCC-3′ Probe: 5′-ACT AAT ACA CCT GTA CAG GCT-3′
HPV 11	Forward: 5′-AAA GAT GCC TCC ACG TCT GCA-3′ Reverse: 5′-ACG ACC CTT CCA CTG GTT ATT-3′ Probe: 5′-CTG CAT ATG CAC CTA CAG TAG-3′	Forward: 5′-GAT ATA GTA CTA GAC CTG CAG-3′ Reverse: 5′-GCC CAG CAA AAG GTC TTG TAG-3′ Probe: 5′-CAG CAA CGT CCG ACT GGT TG-3′	Forward: 5′-GGA ATA CAT GCG CCA TGT G-3′ Reverse: 5′-CGA GCA GAC GTC CGT CCT CG-3′ Probe: 5′-CAG GAT CCC TAT AAG GAT ATG-3′
HPV 16	Forward: 5′-ATG TTT CAG GAC CCA CAG GAG-3′ Reverse: 5′-TGA TGA TCT GCA ACA AGA C-3′ Probe: 5′-TGT TAT AGT TTG TAT GGA ACA-3′	Forward: 5′-CCA GAG ACA ACT GAT CTC TAC-3′ Reverse: 5′-GGT TTC TGA GAA CAG ATG GGG-3′ Probe: 5′-GAC ATT CGT ACT TTG GAA GAC CT-3′	Forward: 5′-CAG ACT ACT TGC AGT TGG-3′ Reverse: 5′-CCA CAC CTG CAT TTG CTG CAT AAG C-3′ Probe: 5′-ACA GGG TAT TTA GAA TAC ATT T-3′
HPV 18	Forward: 5′-ATG GCG CGC TTT GAG GAT CC-3′ Reverse: 5′-TAC TTG TGT TTC TCT GCG TCG-3′ Probe: 5′-TCA GAC TCT GTG TAT GGA GAC-3′	Forward: 5′-CCT AAG GCA ACA TTG CAA GAC A-3′ Reverse: 5′-CTG CTG GGA TGC ACA CCA C-3′ Probe: 5′-GGT TGA CCT TCT ATG TCA-3′	Forward: 5′-CAA TCC TTA TAT ATT AAA GGC ACA GGT ATG-3′ Reverse: 5′-CAT CAT ATT GCC CAG GTA CAG GAG ACT GTG-3′ Probe: 5′-CGT GCT TCA CCT GGC AGC TGT-3′

was 5′-ACA CAA CGT TGT TCA CTA GC-3′. Probe sequences for HPV types 16, 18, 6 or 11 are indicated in Table 1. Incorporation of ^{32}P into each probe was measured and 1.5×10^7 cpm of radiolabeled probe was added to the hybridization vessel containing a pre-hybridized nylon membrane. Hybridization was conducted overnight at 50 °C.

The radiolabeled membranes were washed with 60 ml 6× Saline-Sodium Citrate (SSC) at room temperature three times for 10 min each to remove non-specifically bound probe. Stringent washes were performed to yield optimal signal intensity for the individual probe, at temperatures ranging from 42 and 55 °C, for times from 10 to 20 min. The first wash was performed in 6× SSC, the second in 2× SSC, and the third in 0.5× SSC. Blots were dried and exposures of autoradiographic film were done. Results of each assay were scored from overnight autoradiographs by visual assessment.

For each sample tested, in order to be considered positive for any of the HPV types tested, the sample had to be positive for at least two of the three genes (L1, E6 and E7) and be positive in the human β-globin assay. If a sample was positive in only one gene or did not amplify in the human β-globin assay, a new aliquot of the sample was processed and tested. If upon retest two or three genes were positive or the same single gene from the previous test was positive, the sample was considered positive.

Operating characteristics of the PCR assays were validated through statistical analysis of a series of experiments. A test of extra-binomial variability showed there was no statistically significant impact on the variability due to different operators, instruments or QIAamp plates. Minimum detectable levels were from 6 to 13 copies for the three genes combined for each HPV type. The false positive rate for an individual sample was determined to be 0.26% (upper one-sided 95% confidence bound equal to 0.84%) using the laboratory scoring criterion described above. Clinical assay validation showed the 95% upper confidence bounds for false negativity and false positivity were 0.7 and 0.8%, respectively.

A competitive radioimmunoassays (cRIA) was developed to quantify serum anti-HPV [15]. The cRIA is based on competition between anti-HPV in the participant's serum and a known neutralizing monoclonal anti-HPV16. Details of the assay are described in the accompanying manuscript by Fife et al. The serostatus cutoff was 10 mMU/ml for HPV11 and 6 mMU/ml for HPV16. Test samples exceeding the sero-status cutoff were calibrated against the reference standard. Based on the results of the assay validation experiments, the percentage relative standard deviation (an estimate of the inter- and intra-assay variability of the test) of a sample was estimated to be 36% in the HPV16 cRIA and 23% in the HPV11 cRIA. The cRIAs were used for all immunogenicity evaluations. In addition, serum from each

participant in Protocol 002 was evaluated by HPV16 ELISA at enrollment [20].

2.4. Definition of the efficacy cohort and efficacy analysis

The goal of this post hoc analysis was to assess efficacy of the HPV16 L1 VLP vaccine in preventing HPV16 infection. Combining data from the two protocols was considered appropriate because the protocols had nearly identical inclusion/exclusion criteria, had similar visit schedules, had identical data and sample collection techniques and had samples tested for HPV16 PCR by the same assays.

Protocol 002 HPV16 L1 VLP vaccine-recipients were compared with participants who did not receive HPV16 L1 VLP vaccine, including all Protocol 001 participants, plus the 27 placebo recipients in Protocol 002. Vaccine efficacy was evaluated starting after Month 3 (1 month after the second vaccination) to allow for a longer "at-risk" period for evaluation of new HPV16 infections than would have resulted from evaluating infection after three doses. Starting the efficacy evaluation post-dose 2 was appropriate because anti-HPV16 geometric mean titers (GMTs) post-dose 2 were at least as high as the GMTs during the persistence phase.

Since the HPV L1 VLP vaccines are being developed as prophylactic vaccines, the evaluation of efficacy attempted to include only HPV16-naïve participants (participants who, at the time of enrollment, had not been infected with HPV16). Thus, only participants in Protocol 002 who were HPV16 seronegative at Day 0 (i.e. no antecedent HPV16 infection that resulted in an immune response) and HPV16 PCR negative at Day 0 and Month 3 (i.e. no ongoing HPV16 infection) were included in this analysis.

Participants in Protocol 001 were tested for HPV16 DNA by PCR at Day 0 and Month 3, but were not tested for HPV16 aerostats at Day 0. HPV16 PCR positive participants were excluded from the analysis. All remaining participants were assumed to be HPV16 seronegative at Day 0 for this analysis. HPV16 seropositivity in these HPV-PCR-negative women likely would have indicated past infection with HPV16. These seropositive, PCR negative women would likely have mounted an immune response to HPV16, cleared the infection, and may have been protected against future infection. Inclusion of such women in the analysis would decrease the apparent HPV16 infection rate in the part of the control group derived from Protocol 001. The net effect of this assumption was a conservative estimate of vaccine efficacy.

Since the efficacy evaluation started after Month 3, specimens collected at Month 7 and later were considered part of the efficacy evaluation phase of the study, and were therefore used to identify cases of new HPV16 infection. A case of HPV16 infection was defined as at least 1 positive PCR result at two or more consecutive visits spaced at least 4 months (120 days) apart, or a positive PCR result at the last visit on record for the participant (Table 2). All other scenarios were considered to be non-cases of HPV16 infection.

Table 2
Case definitions of HPV16 infection[a]

Month 7	Month 12	Month 24	Month 36
+	+	+/−/NA	+/−/NA
+/−/NA	+	+	+/−/NA
+/−/NA	+/−/NA	+	+
+	NA	NA	NA
+/−/NA	+	NA	NA
+/−/NA	+/−/NA	+	NA
+/−/NA	+/−/NA	+/−/NA	+

NA: PCR results missing at the given time point; +: HPV16 PCR positive on any sample at the given time point; −: LHPV16 PCR negative on all samples at the given time point.

[a] This table contains all possible ways in which a participant can be defined as having a case of HPV16 infection.

With respect to missing data, following Month 3, if more than two of the required PCR samples were missing results at any time point, the visit was considered missing. If up to two results were missing, the visit was included in the analysis. By incorporating incomplete data into the analysis, there was a slight potential for undercounting cases of HPV infection. For example, a participant with two of four samples negative and two of four samples missing at Month 24 was defined as being PCR negative at Month 24 even though it was possible that one of the missing samples was positive.

Person-years were used as the unit for the follow-up time. Since the efficacy evaluation began after Month 3, the person-years of follow-up for each participant were determined by computing the number of days between the participant's Month 3 visit and the participant's last follow-up date (for non-cases) or the date the participant became a case (for cases) and dividing this number by 365.25.

A point estimate of the vaccine efficacy and the corresponding 95% two-sided confidence interval were provided. An exact conditional procedure was used under the assumption that the numbers of cases in the vaccine group (C_v) and in the control group (C_p) are independent Poisson random variables with means λ_v and λ_p, respectively [21]. The number of cases in the vaccine group C_v, given the total number of cases observed $C_v + C_p$, is binomially distributed with parameters, $C_v + C_p$, and $p = \lambda_v/(\lambda_v + \lambda_p)$, where p is the probability that a subject in the vaccine group is a case. The point estimate for p is $C_v/(C_v + C_p)$. The lower bound of the $100(1 - \alpha)\%$ exact confidence interval for p can be calculated by searching for the p_L such that the probability of observing C_v or more vaccine cases out of $C_v + C_p$ total cases is $\alpha/2$. The upper bound of the exact confidence interval for p can be calculated by searching for p_U such that the probability of observing C_v or fewer vaccine cases out of $C_v + C_p$ total cases is $\alpha/2$. The estimate of the vaccine efficacy must account for the follow-up in each group. Therefore, the point estimate for the vaccine efficacy was calculated using the formula $VE = 1 - (C_v/k_v)/(C_p/k_p)$, where k_v and k_p are the person-years of follow-up in the vaccine and control groups, respectively. The following formulas were used for the endpoints of the confidence interval for the

vaccine efficacy: lower bound $VE_L = (1 - p_U/k)/(1 - p_U)$ and upper bound $VE_U = (1 - p_L/k)/(1 - p_L)$, where $k = k_v/(k_v + k_p)$, follow-up in vaccine group/total follow-up.

To assess whether or not the study cohorts may have differed in their exposure to HPV, the infection rates for another high risk type (HPV18) were compared between the two protocols, since none of the participants in either study was vaccinated against HPV18. Infection rates for HPV18 were calculated for Protocols 001 and 002 separately to assess qualitatively if they were comparable. Infection rates for HPV18 also were calculated for the HPV16 L1 VLP vaccine recipients in Protocol 002 and the control group (placebo recipients in Protocol 002 and all of the Protocol 001 participants) separately to assess whether the efficacy comparison groups were comparable. The infection rates were adjusted for the person-years of follow-up in each protocol.

To be included in this analysis, participants had to be HPV18 PCR negative at Day 0. A case of HPV18 infection was defined as one or more positive PCR result at any visit after Day 0. All of the analyses were pre-specified prior to analyzing the data with the exception of the rules for handling missing data. This analysis was post hoc and unblinded to the treatment assignments and protocol of the participants. Therefore, there was a potential for bias in the conduct of the analysis. The two protocols were combined because of similar study designs. The two protocols were conducted in different locations and were intended primarily as Phase I immunogenicity studies. A small number of cases of HPV16 infection were expected because of the small sample sizes of the studies and the limited duration of follow-up. This limited the statistical power of the analysis.

3. Results

3.1. Study participant characteristics and accounting

Age, race and smoking status by treatment group (HPV16 L1 VLP vaccine or control) are displayed in Table 3. Overall, 249 women were enrolled: 167 in the control group and 82 in the HPV16 L1 VLP vaccine group. The median ages and racial distribution of the two groups were similar. There was a higher percentage of smokers in the control group (32%) than in the vaccine group (12%).

The reasons for exclusion from the efficacy analysis of the HPV16 L1 VLP vaccine are summarized in Table 4. Subjects with multiple reasons for exclusion were counted under each reason. One hundred and ninety-five participants out of 249 were included in this analysis of which 129 were in the control group and 66 were in the vaccine group. Twenty-five participants in the control group (24 from Protocol 001 and 1 from Protocol 002) discontinued the study during the vaccination phase, while 5 participants in the HPV16 L1 VLP vaccine group discontinued the study during the vaccination phase. All participants in Protocol 001 were assumed to be HPV16 seronegative at Day 0. Besides discontinuation from the study, the most common reasons for exclusion were HPV16 seropositivity at Day 0, missing three or more HPV16 PCR results at Day 0 and/or Month 3, and HPV16 positivity by PCR at baseline.

Table 3
Summary of participant characteristics

	Treatment group		
	Placebo or HPV11 L1 VLP vaccine	HPV16 L1 VLP vaccine	Total
Participants vaccinated	167	82	249
Age (years)			
Median	20	20	20
Range	18–26	18–25	18–26
Race/ethnic group			
Asian	12 (7%)	10 (12%)	22 (9%)
Black	6 (4%)	2 (2%)	8 (3%)
Caucasian	135 (81%)	65 (79%)	200 (80%)
Hispanic	13 (8%)	1 (1%)	14 (6%)
Other	1 (1%)	4 (5%)	5 (2%)
Smoking status			
Non-smoker	114 (68%)	72 (88%)	186 (75%)
Smoker	53 (32%)	10 (12%)	63 (25%)

3.2. Vaccine tolerability

No serious vaccine-related adverse experiences occurred in any participant in either Protocol. Most adverse experiences were mild to moderate in intensity. The most common injection site and systemic adverse experiences were pain/tenderness/soreness and headache, respectively (see accompanying manuscript, Fife et al.).

Table 4
Participants excluded from the efficacy analysis of the vaccine

	Treatment group	
	Control ($N = 167$)	HPV16 L1 VLP vaccine ($N = 82$)
Participants included in the analysis	129	66
Participants excluded from the analysis because:	38	16
HPV16 seropositive at Day 0	2	10
Missing ≥3 HPV16 PCR results (Day 0 or Month 3)	9	3
HPV16 positive by PCR at baseline[a]	14	2
Missed Month 2 injection	2	2
Discontinued the study[b]	25	5

All Protocol 001 participants were assumed to be HPV16 seronegative at Day 0. Participants with multiple exclusions are included in more than one category. Thus, the categories are not mutually exclusive.

[a] HPV16 PCR negative = negative by PCR at Day 0 and Month 3 on all cervicovaginal swabs. HPV16 PCR positive = positive by a PCR on ≥1 swab for ≥1 time point.

[b] During vaccination phase.

Table 5
Summary of cases of HPV16 infection

Analysis	Control/placebo			HPV16 L1 VLP vaccine		
	No. of cases	No. of subjects	Person-years	No. of cases	No. of subjects	Person-years
Protocols 001 + 002 combined	15	129	298.1	0	66	173.1

3.3. Anti-HPV16 immune responses

All participants who received active HPV16 L1 VLP vaccine developed a detectable antibody response. Anti-HPV16 responses in Protocol 002 were highest 1 month post-dose 3, and were highest in the 80 μg dosage group (see accompanying manuscript, Fife et al.).

3.4. Efficacy analysis

There were 15 cases of HPV16 infection. All 15 cases occurred in control group participants. Of these 15 cases, 13 occurred in Protocol 001 (3 who had received placebo and 10 who had received the HPV11 vaccine), and 2 occurred in Protocol 002 (both placebo recipients). Of the 15 participants who became HPV16 infected, 5 had at least 2 years of follow-up, 3 had at least 1 year but less than 2 years of follow-up and 7 had less than 1 year of follow-up. Of the 15 cases of HPV16 infection, there was one case based on PCR positivity on four consecutive visits, two were cases of PCR positivity on three consecutive visits, six were cases of PCR positivity on two consecutive visits, and six were cases of PCR positivity at the last visit only. No participant exhibited intermittent HPV16 DNA positivity. Three participants in the control group and none in the vaccine group exhibited single PCR positivity at a visit other than their last visit.

Table 5 displays the number of cases of HPV16 infection by treatment group for Protocols 001 and 002 combined. The number of participants and total person-years of follow-up in each treatment group are also provided. For the combined analysis, the estimate of the true vaccine efficacy was 100% and the 95% confidence interval for the true vaccine efficacy was (52, 100%). The wide confidence interval observed with this analysis is a function of the small sample size and number of cases.

In Protocols 001 and 002 combined, the incidence rates of HPV16 infection were 0 events per 100 person-years of follow-up in the vaccine group and 5.0 events per 100 person-years of follow-up in the control group.

3.5. Analysis of HPV18 infection

There were a total of 26 cases of HPV18 infection of which 13 were in Protocol 001 (11 received HPV11 vaccine and 2 received placebo) and 13 were in Protocol 002 (9 received HPV16 L1 VLP vaccine and 4 received placebo). Table 6 displays a summary of the cases of HPV18 single positivity by protocol and treatment group. The total person-years of follow-up, the mean person-years of follow-up, and the median person-years of follow-up were similar between the two protocols. The HPV18 incidence rate was 4.6 per 100 person-years in Protocol 001, and 4.9 per 100 person-years in Protocol 002. The HPV18 incidence rate was 4.5 per 100 person-years in the HPV16 vaccine group, and 4.9 per 100 person-years in the control group. These results show that the incidence of HPV18 infection, an infection that is expected to be indifferent to the vaccines used in either protocol, was comparable between the two protocols and the two efficacy comparison groups. Therefore, the exposure to HPV was approximately equal in the two protocols and the two efficacy comparison groups. A reciprocal analysis for HPV 11 was not done because there were extremely few cases of HPV 11 infection 0 cases in Protocol 001 and 2 cases in Protocol 002 (both in the vaccine group).

4. Discussion

In this post hoc combined analysis of two vaccine protocols, we found evidence that the HPV16 L1 VLP vaccine reduced the incidence of HPV16 infection. Although the primary reason to vaccinate against HPV16 infection is to prevent cervical cancer, this endpoint would be difficult to study for ethical and scientific reasons. HPV16 infection is a reasonable surrogate endpoint since approximately 50% of cervical cancers are associated with this type [1]. The current analysis provides supportive evidence for a recent study that demonstrated a protective effect of the HPV16 L1 VLP vaccine in a placebo-controlled protocol [19]. In

Table 6
Summary of cases of HPV18 single positivity by protocol and treatment group

	Placebo			Vaccine		
	No. of cases	No. of participants	Person-years	No. of cases	No. of participants	Person-years
Protocol 001	2	26	60.3	11	106	223.6
Protocol 002	4	27	65.6	9	80	200.7

that study, 2392 women (16–23 years old) were randomized to receive placebo or the HPV16 L1 VLP vaccine. The incidence of persistent HPV16 infection was 3.8/100 participant-years at risk in the placebo group and 0.0/100 participant-years at risk in the vaccine group (100% efficacy, 95% confidence interval, 90–100%; $P < 0.0001$). All nine cases of HPV16-related cervical intraepithelial neoplasia occurred among placebo recipients.

In the current study, all women who received the three dose regimen of HPV16 L1 VLP vaccine developed an antibody titer suggesting seroconversion. At Month 7, the anti-HPV16 GMT was several-fold higher than the anti-HPV16 GMT observed for women with serologic evidence of natural HPV16 infection at enrollment. Similar results were obtained in an immunogenicity study of a baculovirus-derived HPV16 L1 VLP vaccine [18]. The duration of antibodies and protection remains to be determined.

In summary, this analysis suggests a protective effect of the HPV16 L1 VLP against HPV16 infection. Further large-scale studies are in progress that will define antibody persistence and the true efficacy of HPV L1 VLP vaccines in preventing infection and clinical disease.

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