Effect of Human Papillomavirus 16/18 L1 Viruslike Particle Vaccine Among Young Women With Preexisting Infection

A Randomized Trial

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Context Viruslike particle human papillomavirus (HPV) vaccines were designed to prevent HPV infection and development of cervical precancers and cancer. Women with oncogenic HPV infections might consider vaccination as therapy.

Objective To determine whether vaccination against HPV types 16 and 18 increases the rate of viral clearance in women already infected with HPV.

Design and Setting Phase 3, masked, community-based randomized trial conducted in 2 provinces of Costa Rica.

Participants A total of 2189 women aged 18 to 25 years who were recruited between June 2004 and December 2005. Participants were positive for HPV DNA at enrollment, had at least 6 months of follow-up, and had follow-up HPV DNA results.

Intervention Participants were randomly assigned to receive 3 doses of a bivalent HPV-16/18 L1 protein viruslike particle AS04 candidate vaccine (n=1088) or a control hepatitis A vaccine (n=1101) over 6 months.

Main Outcome Measures Presence of HPV DNA was determined in cervical specimins by a molecular hybridization assay using chemiluminescence with HPV RNA probes and by polymerase chain reaction using SPF10 primers and a line probe assay detection system before vaccination and by polymerase chain reaction after vaccination. We compared rates of type-specific viral clearance using generalized estimating equations methods at the 6-month visit (after 2 doses) and 12-month visit (after 3 doses) in the 2 study groups.

Results There was no evidence of increased viral clearance at 6 or 12 months in the group who received HPV vaccine compared with the control group. Clearance rates for HPV-16/18 infections at 6 months were 33.4% (82/248) in the HPV vaccine group and 31.6% (95/298) in the control group (vaccine efficacy for viral clearance, 2.5%; 95% confidence interval, –9.8% to 13.5%). Human papillomavirus 16/18 clearance rates at 12 months were 48.8% (86/177) in the HPV vaccine group and 49.8% (110/220) in the control group (vaccine efficacy for viral clearance, –2.0%; 95% confidence interval, –24.3% to 16.3%). There was no evidence of a therapeutic effect for other oncogenic or nononcogenic HPV categories, among women receiving all vaccine doses, among women with single infections, or among women stratified by the following entry variables: HPV-16/18 serology, cytologic results, HPV DNA viral load, time since sexual debut, *Chlamydia trachomatis* or *Neisseria gonorrhoeae* infection, hormonal contraceptive use, or smoking.

Conclusion In women positive for HPV DNA, HPV-16/18 vaccination does not accelerate clearance of the virus and should not be used to treat prevalent infections.

Trial Registration clinicaltrials.gov Identifier: NCT00128661

JAMA. 2007;298(7):743-753 www.jama.com

Knowledge that infection with 1 of approximately 15 oncogenic human papillomavirus (HPV) types is required for the development of cervical cancer has permitted primary prevention efforts via vaccination.[1] Two vaccines based on HPV L1 protein viruslike particles (VLPs) are undergoing evaluation in large-scale clinical trials.[2-5] One vaccine (Gardasil) is a quadrivalent HPV-16/18 cervical cancer candidate vaccine that contains VLPs from 2 oncogenic HPV types, HPV-16 and HPV-18, and also contains VLPs from HPV types 6 and 11, which are not involved in cervical cancer pathogenesis but are linked to benign genital warts. This vaccine has been approved

For editorial comment see p 805.

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for use in women aged 9 to 26 years in the United States and several other countries. The second vaccine (Cervarix) is a bivalent HPV-16/18 cervical cancer candidate vaccine that contains VLPs only from the 2 oncogenic HPV types, HPV-16 and HPV-18. This vaccine has been approved for use in Australia. Application for approval in the United States and other countries is under review by the US Food and Drug Administration and other regulatory bodies.

Viruslike particle–based vaccines have been shown to provide near complete type-specific protection against infection with HPV types included in the vaccine in the initial years following vaccination.[2,3,6] Preliminary evidence suggests that at least 1 of the vaccines (the bivalent HPV-16/18 cervical cancer candidate vaccine) might provide partial protection against oncogenic HPV types phylogenetically related to HPV-16/18 (ie, HPV types 31 and 45).[3] The protection against initial infection afforded by vaccination with either of the vaccines under evaluation is believed to be mediated primarily by neutralizing antibodies generated through vaccination.[7,8] Consistent with this concept, high levels of antibodies are observed systemically and in the genital tract after vaccination in humans.[9-11]

Antibodies are not typically involved in treating intracellular infections after their establishment; however, HPV vaccination has also been shown to induce cell-mediated immune responses traditionally involved in the eradication of infections.[10,12-15] If directed against the appropriate antigenic targets, these cell-mediated responses could provide some benefit of vaccination among individuals already infected. Given that HPV infection depends on the viral genome being present in the epithelial basal cells that give rise to the cells in the higher layers of the epithelium and that the L1 protein is only expressed in these higher cell layers, it is not clear whether the vaccine-induced immune response directed against L1 would have curative potential among infected individuals.[8] Animal studies have not supported such therapeutic effects of HPV VLP-based vaccination, but no published data on humans exist that directly address this possibility.[16]

Most HPV infections, regardless of type, clear spontaneously, typically within 6 months to 2 years.[17] Risk of progression to in situ disease and invasive cancer is highest among the small subset of women with persistent infections beyond this period.[1] Understanding whether vaccination provides any therapeutic benefit to infected women is of importance in countries where HPV DNA testing has been incorporated into cervical cancer screening programs.[18,19] Women in such programs who test positive for HPV might want to avail themselves of the vaccine instead of waiting several months to determine whether their infections clear or opting for treatments based on excision or ablation of the cervical transformation zone where cancers arise.

Because current management protocols often involve retesting HPV-positive women within months of an initial HPV-positive result before treatment decisions are made, understanding the impact of vaccination on viral clearance in the first 6 to 12 months following an initial HPV-positive result would be informative. If effective at clearing established infections, it is reasonable to expect that vaccination would work within this time frame, given the near complete rate of seroconversion and high levels of immune response observed after 1 or 2 doses of vaccine.[10-12]

To directly address the question of whether women positive for HPV DNA should be encouraged to receive HPV-16/18 vaccination as a useful strategy to induce or accelerate clearance of their infections, we evaluated whether the rates of resolution of prevalent HPV infections are affected by vaccination in an ongoing community-based randomized clinical trial conducted in Costa Rica.

METHODS

Recruitment and Eligibility

Women included in the present evaluation are participants in a larger, ongoing randomized clinical trial of 7466 women designed to evaluate the efficacy of an HPV-16/18 VLP vaccine formulated with the AS04 adjuvant system (Cervarix, GlaxoSmithKline [GSK] Biologicals, Rixensart, Belgium) against persistent type-specific infection with HPV and HPV-associated precancerous lesions. Participants in our clinical trial were women aged 18 to 25 years residing in the provinces of Guanacaste and Puntarenas in Costa Rica who were identified via a new population census. Women identified through this census were invited to participate via a letter delivered to their home by study staff members. Women who attended 1 of the 7 study clinics were screened for eligibility between June 28, 2004, and December 21, 2005.

To be eligible for study, women had to fulfill the following requirements at the time of entry: age 18 to 25 years (inclusive), planned residence in the study area for the 6 months following enrollment, ability to speak/understand Spanish, good general health as determined by history and a physical examination, and willingness to provide written informed consent.

Specific exclusion criteria at enrollment included history of chronic or immunodeficient conditions requiring treatment; history of allergic reaction to any vaccine or of significant allergic conditions, suspected allergy, or reactions to components of the vaccine or to latex; history of vaccination against hepatitis A or a known history of hepatitis A infection; history of recent (≤6 months) long-term administration of immunosuppressants or immune-modulating drugs; and unwillingness to use an effective method of birth control for a period covering the vaccination phase of the trial (among sexually active individuals). Enrollment of pregnant women was deferred until they were at least 3

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months post partum and no longer breastfeeding. No screening for HPV DNA or antibodies was performed before enrollment/vaccination. The trial was reviewed and approved by human subjects review committees of the National Cancer Institute in the United States and INCIENSA (Instituto Costarriciense de Investigacion y Ensenanza en Nutricion y Salud) in Costa Rica.

Data and Specimen Collection Prior to Randomization/Vaccination

A risk factor questionnaire was administered to all eligible participants who consented to participation. A pelvic examination was performed on all sexually experienced women, at which time exfoliated cells were collected in Preservcyt liquid medium (Cytyc Corp, Marlborough, Massachusetts) for Thinprep (Cytec Corp) cytologic evaluation and for HPV DNA, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* testing. Blood specimens were also collected from all participants prior to randomization and vaccination; serum samples obtained from these specimens were used for HPV antibody testing.

Randomization/Vaccination

Women in our trial were randomized at the site in a blinded fashion to receive either the HPV-16/18 VLP vaccine formulated with the AS04 adjuvant system or a control hepatitis A vaccine consisting of inactivated viral antigen formulated with alum (Havrix, GSK Biologicals). Study and control vaccines were assigned random vaccine identification numbers at the time of labeling by the manufacturer. Study personnel at the Costa Rican study site randomized participants by assigning each eligible participant to the next available sequential vaccine identification number. The protocol called for a dose of vaccine at each of 3 study visits: at enrollment, 1 month following the initial dose (allowable range, 21-120 days), and 6 months following the initial dose (allowable range, 121-300 days).

At the 6-month clinic visit, all sexually experienced women were instructed to self-collect a cervicovaginal specimen using a Dacron swab. Exfoliated cells from this collection were stored in Preservcyt solution and used for HPV DNA testing. Following this self-administered collection, women who at study entry had evidence of either atypical squamous cells of uncertain significance that were positive for HPV by a molecular hybridization assay using chemiluminescence with HPV RNA probes (the Hybrid Capture 2 [HC2] test; Digene Corp, Silver Spring, Maryland) or a low-grade squamous intraepithelial lesion had a pelvic examination performed. At the time of this pelvic examination, a cervical specimen was also collected by a clinician and stored in Preservcyt solution.

Follow-up

Following the initial vaccination phase, all participants were asked to attend one of the study clinics approximately 1 year after enrollment (12-month visit). At the time of this annual screening visit, a pelvic examination was performed (on sexually active women) and exfoliated cells were collected by a clinician for cytologic evaluation and HPV DNA testing in a manner similar to that described for enrollment. Additional follow-up of participants beyond the 12-month period is ongoing.

Safety Monitoring

Participants remained at the clinic for 30 to 60 minutes following each vaccination. Reactogenicity and adverse event experiences were collected from all participants during this period and in the week following each vaccination via home visits for a 10% randomly selected sample of participants. Adverse event and pregnancy information was also actively collected from all participants at each of the follow-up clinic visits. In addition, a toll-free number is continuously staffed by clinical personnel, and participants are instructed to call this number between visits if they experience any medical event.

A data and safety monitoring board (DSMB) established by the National Cancer Institute to oversee the trial meets on a regular basis to evaluate safety data in closed session (date of most recent meeting: June 19, 2007). The DSMB has recommended trial continuation. The trial statistician (S.W.) participates in the closed-session review of summary tables of adverse events by group but is not involved in the formulation of the final DSMB recommendations. Since the ongoing main trial is still blinded, other investigators and site personnel have no access to safety data by treatment group; therefore, no safety data are presented herein. Safety data and the main prophylactic efficacy results will be reported once the final analysis for the main trial is begun.

HPV DNA Testing

Human papillomavirus DNA testing was performed on enrollment (prevaccination) specimens using a 2-mL aliquot of exfoliated cells stored in Preservcyt solution. Testing was performed using probe B (designed to detect 13 oncogenic HPV types including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) per manufacturer's instructions in a laboratory located at the University of Costa Rica in San Jose.[20]

Broad-spectrum polymerase chain reaction (PCR)–based HPV DNA testing was performed at Delft Diagnostics Laboratory (Delft, the Netherlands) using a previously described procedure based on amplification using the SPF10 primers and a DNA enzyme immunoassay detection of amplimers and, if positive, followed by typing using the line probe assay (LiPA) line blot detection system (Inno-LiPA HPV genotyping assay SPF10 system, version 1, Labo Bio-medical Products, Rijswijk, the Netherlands).[21-23] The LiPA assay detects 25 HPV genotypes (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74). Testing was performed on a 0.5-mL aliquot of Preservcyt solution removed from the cytologic specimen prior to slide

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preparation to reduce the risk of carryover. In addition, using DNA extracted from the same aliquot, and to ensure that HPV-16 and HPV-18 infections were not missed, all specimens that screened positive for HPV DNA using SPF10 DNA enzyme immunoassay but were negative for HPV-16 or HPV-18 by LiPA were tested for the presence of HPV-16 and HPV-18 DNA using type-specific primers, as previously described.[23,24]

Enrollment specimens (prevaccination) and specimens collected at the 6-month and 12-month visits were tested by the PCR method. For the enrollment and 12-month visits, cervical specimens collected during the pelvic examination were used. For the 6-month visit, self-collected cervicovaginal specimens were used. In addition, for the subset of women who had a pelvic examination performed at the time of the 6-month visit, cervical specimens collected during the pelvic examination were used for PCR-based HPV DNA testing.

For 672 women with both self- and clinician-collected exfoliated specimens at the 6-month visit, a high degree of concordance was observed in HPV results from these 2 specimen types. Agreement and κ values for HPV detection between the self-collected and clinician-collected specimens were 96.0% and $\kappa=0.86$ (McNemar $P=.56$, giving no indication of directionality) for HPV-16 and 97.6% and $\kappa=0.81$ (McNemar $P>.99$) for HPV-18. Overall agreement (all types) was 89.4% with $\kappa=0.59$ (McNemar $P=.19$). Given this high level of agreement, HPV testing results using the self-collected specimens were used to define HPV status at the 6-month visit.

HPV-16/18 Antibody Testing

An enzyme-linked immunosorbent assay was used to test serum specimens collected from participants at entry (prevaccination) for antibodies against HPV-16 and HPV-18, using previously described methods.[10] Testing was performed at GSK Biologicals in Rixensart, Belgium.

Chlamydia trachomatis and *Neisseria gonorrhoeae* DNA Testing

Presence of *C trachomatis* and *N gonorrhoeae* infection was determined by testing for the presence of DNA from these pathogens in a 2-mL aliquot of exfoliated cells collected at entry (prevaccination) and stored in Preservcyt solution. The HC2 method was used per manufacturer's instructions.[25] Testing was performed in a laboratory located at the University of Costa Rica.

Statistical Analysis

Data analyses were directed by the trial co–principal investigators (A.H. and R.H.) and the trial statistician (S.W.). The analyses were performed by programming staff at the trial's data management center (Information Management Services Inc, Silver Spring, Maryland) under direct contract and supervision by the National Cancer Institute and handled according to standard operating procedures that ensure maintenance of blinding and overall trial integrity. Investigators, trial staff, and participants were unaware of individual participants' vaccine group assignment.

Comparisons between study groups with respect to general characteristics were made using the χ^2 test for categorical variables and the Wilcoxon test for continuous variables. Among infected women, 42.6% had more than 1 HPV type at enrollment. We chose to use an infection rather than a woman as the unit of analysis because of our interest in clearance of individual HPV types.

We evaluated the following HPV categories: HPV-16; HPV-18; HPV-16/18 (HPV-16 and/or HPV-18); HPV types from the alpha-9 species, excluding HPV-16 (HPV types 31, 33, 35, 52, and 58); HPV types from the alpha-7 species, excluding HPV-18 (HPV types 39, 45, 59, and 70; HPV-68 was not considered in this category because LiPA cannot differentiate HPV-68 from HPV-73); oncogenic HPV types other than those from the alpha-7 and alpha-9 species (HPV types 51, 56, 66, and 68/73); and nononcogenic HPV types (HPV types 6, 11, 34, 40, 42, 43, 44, 53, 54, and 74). In addition, we evaluated HPV positivity by the HC2 test at entry, as evaluation of this group is relevant from a clinical management perspective.

Percentage clearance was evaluated independently at the 6-month visit (after 2 doses of vaccine) and at the 12-month visit (after 3 doses of vaccine). Viral clearance of a specific HPV type was defined as failure to detect at the 6-month or 12-month visit an HPV type that was present before vaccination. For the analysis of viral clearance among women who were HC2-positive at entry, viral clearance was defined as absence at the 6-month or 12-month visits of HPV types detected at entry by type-specific PCR-based testing. Those who were positive at entry or follow-up by HC2 analysis but negative for all specific types tested (ie, HC2-positive/SPF10-positive/LiPA-negative) were considered to be positive for an unknown type.

Women who tested positive for an unknown type at entry and follow-up were considered to have a persistent infection (n=13 or 0.5% of all infections at 6 months; n=13 or 0.7% of all infections at 12 months). Women positive at entry for an unknown type who tested positive for a known HPV type at the follow-up visit were considered to have cleared their initial infection and acquired a new one (n=46 or 1.7% of all infections at 6 months; n=31 or 1.5% of all infections at 12 months). Similarly, women positive at entry for a known type who tested positive for an unknown type at the follow-up visit were considered to have cleared their initial infection and acquired a new one (n=89 or 3.3% of all infections at 6 months; n=98 or 4.9% of all infections at 12 months).

Vaccine efficacy for viral clearance (VEVC), a measure of the percentage reduction (or increase) in infection rates observed when the HPV vaccine group

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is compared with the control group, was defined as

(Pr [viral persistence in control group] – Pr [viral persistence in HPV vaccine group])/Pr (viral persistence in control group) =

1 – (Pr [viral persistence in HPV vaccine group]/Pr [viral persistence in control group]) =

1 – RR,

where Pr represents the probability of persistence at the time point of interest (6 or 12 months) and RR is the ratio of the probability (risk) of persistence in the 2 groups. Ninety-five percent confidence intervals (CIs) around VEVC estimates were computed from the CIs for the RR.

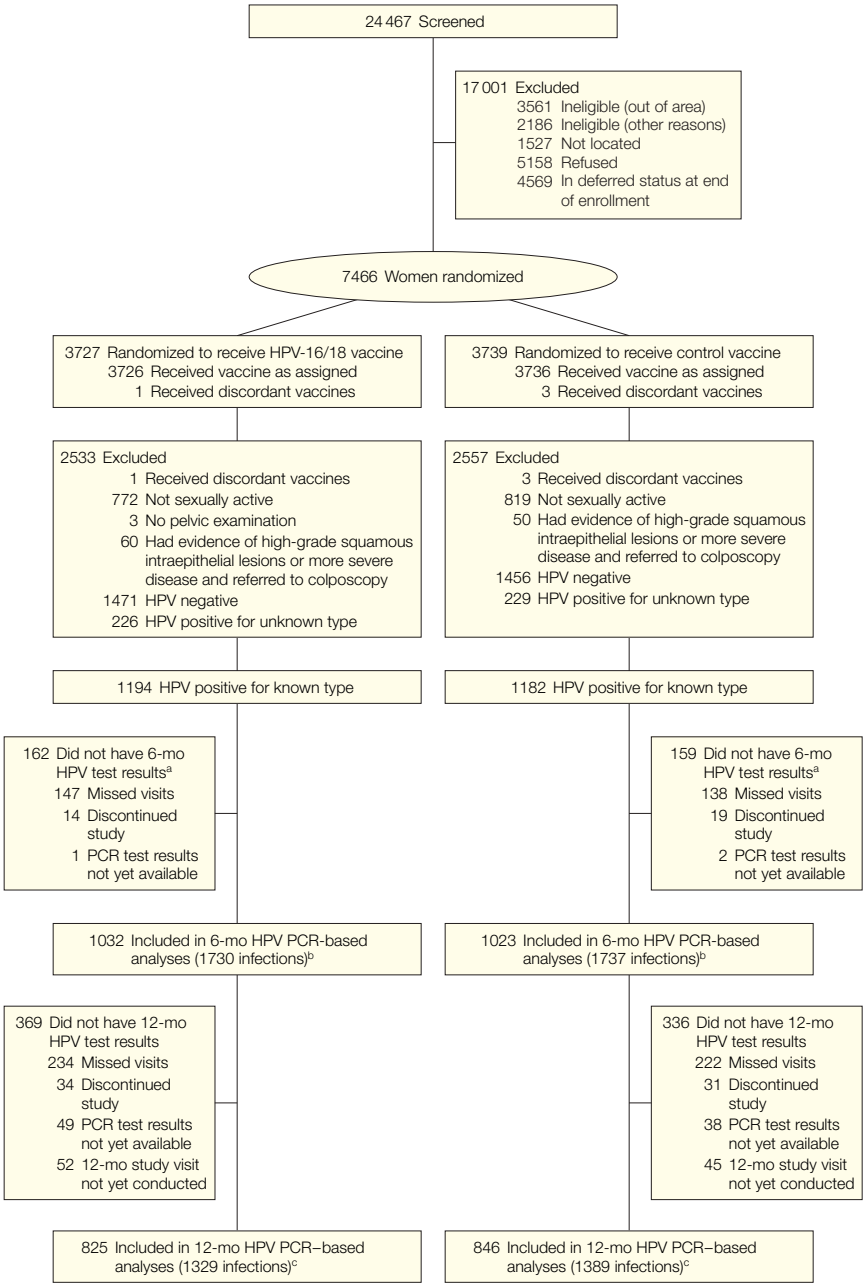
The generalized estimating equations method was used to account for possible lack of independence between clearance in analysis of more than 1 infection in the same woman.[26] The estimates of the proportion of infections that clear from the generalized estimating equations analysis can, therefore, be slightly different from the crude percentages. We present VEVC against persistence for several HPV categories at the 6-month and 12-month visits overall as well as restricted to women who received all vaccine doses and to those with evidence of a single HPV type at entry. Additional analyses were performed to evaluate VEVC stratified by the following entry parameters of interest: HPV-16/18 antibodies (positive for either vs negative for both), cytologic findings (normal vs atypical squamous cells of uncertain significance), HC2 viral load (relative light unit values, 0-<2.0, 2.0-<50, and ≥50), months since sexual debut (0-36 months, 37-72 months, and ≥73 months), oral or injectable contraceptive use (current users vs not current users), cigarette smoking (current smokers vs not current smokers), and chlamydia/gonorrhea findings (positive for either vs negative for both).

RESULTS

Study Population

A total of 7466 women were enrolled and randomized (FIGURE). For the present evaluation, we excluded 4 women who inadvertently received both vaccine types. Among the remaining 7462 women, 3726 were randomized to the HPV vaccine group and 3736 to the control group. A total of 1594 women (775 in the HPV vaccine group and 819

Figure. Participant Flow



HPV indicates human papillomavirus; PCR, polymerase chain reaction.
[a] Results not available at the time of data freeze (data freeze dates: March 29, 2007, for clinical database; June 26, 2006, for risk factor questionnaire database; April 25, 2007, for HPV PCR database; and July 17, 2006, for HPV-16/18 serology database).
[b] Analysis of clearance of Hybrid Capture 2–positive infections detected at entry were based on 666 women in the HPV vaccine group and 663 women in the control group, as described in the "Methods" section of the text.
[c] Includes 134 women (56 in the HPV vaccine group and 78 in the control group) who did not have HPV PCR results available from the 6-month visit.

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in the control group) were not included in the present evaluation because they were not sexually experienced at enrollment (n=1591) or because a pelvic examination could not be performed for other reasons (n=3). An additional 110 women (60 in the HPV vaccine group and 50 in the control group) were excluded because of evidence of high-grade squamous intraepithelial lesions or more severe disease at study entry that resulted in a referral to colposcopy for evaluation and possible treatment. This group was excluded to avoid any effect of excisional treatment on viral persistence.

Of the 5758 participants remaining, 2376 (41.3%; 1194 in the HPV vaccine group and 1182 in the control group) were positive for HPV DNA at study entry by SPF10/LiPA or by the type-specific HPV-16/18 primer PCR. Individual typing was not obtainable for an additional 455 women (7.9%) (ie, SPF10-positive/LiPA-negative and negative by type-specific HPV-16/18 primer PCR); these women were not included in the main analyses because evaluation of viral clearance requires knowledge of the specific HPV types involved. Of the 2376 women positive for HPV by SPF-10/LiPA at entry, 321 (162 in the HPV vaccine group and 159 in the control group) were excluded from the main analyses because they did not have HPV PCR results available from their 6-month visit. Polymerase chain reaction results were missing for these 321 women because of missed visits (n=285) or study discontinuations (n=33) or because PCR results were not yet available from the testing laboratory (n=3).

Compared with women included in the analysis, the 321 excluded women tended to be slightly younger (mean age, 20.6 years for those excluded vs 21.3 years for those included; $P<.001$) but were comparable with respect to mean lifetime number of sexual partners ($P=.12$), frequency of abnormal cytologic findings at entry ($P=.64$), proportion of individuals with multiple infections ($P=.16$), and distribution of HPV analysis groups ($P=.29$). A lower rate of referral to colposcopy after entry was observed among those excluded from the analysis (5.9% vs 15.7% among those included; $P<.001$). These 321 women were comparable by group with respect to age ($P=.34$), lifetime number of sexual partners ($P=.54$), proportion of participants referred for colposcopy during follow-up ($P=.25$), proportion of individuals with multiple infections ($P=.70$), and distribution of HPV analysis groups ($P=.25$). A small increase in the rate of abnormal cytology at entry was observed in the HPV vaccine group (36.6% vs 25.2%; $P=.03$).

The final number of women in the HPV PCR–based analyses was 2055 (1032 in the HPV vaccine group and 1023 in the control group; $P=.84$). These 2055 women had a total of 3467 infections at entry (1730 in the HPV vaccine group and 1737 in the control

Table 1. Participant Characteristics[a]

Characteristics	HPV Vaccine Group (n = 1088)	Control Group (n = 1101)
Age at entry, y		
18-19	288 (26.5)	313 (28.4)
20-21	295 (27.1)	278 (25.2)
22-23	263 (24.2)	284 (25.8)
24-25	241 (22.2)	226 (20.5)
Lifetime No. of sexual partners		
1	333 (30.6)	325 (29.7)
2	298 (27.4)	315 (28.8)
≥3	456 (42.0)	455 (41.6)
Costa Rican cytologic findings at entry		
Normal	755 (69.6)	796 (72.6)
Abnormal	329 (30.4)	301 (27.4)
Referral to colposcopy after entry[b]		
No	926 (85.1)	941 (85.5)
Yes	162 (14.9)	160 (14.5)
HPV infection status		
Single infection	642 (59.0)	614 (55.8)
Multiple infections	446 (41.0)	487 (44.2)
HPV analysis group[c]		
HPV-16	181 (10.0)	232 (12.3)
HPV-18	81 (4.5)	81 (4.3)
Species alpha-7 excluding HPV-18	257 (14.2)	278 (14.8)
Species alpha-9 excluding HPV-16	444 (24.5)	455 (24.2)
Other oncogenic HPV	427 (23.5)	432 (23.0)
Nononcogenic HPV	425 (23.4)	403 (21.4)
Vaccination doses received		
Dose 1	1088 (100.0)	1101 (100.0)
Dose 2	1034 (95.0)	1035 (94.0)
Dose 3	985 (90.5)	977 (88.7)
All 3 doses	954 (87.7)	950 (86.3)
Follow-up time, median (interquartile range), d		
From entry to 1-mo visit (n = 1034/1035)	36 (33-49)	38 (33-49)
From entry to 6-mo visit (n = 1032/1023)	177 (163-199)	175 (162-200)
From entry to 12-mo visit (n = 825/846)	365 (319-398)	365 (321-398)

Abbreviation: HPV, human papillomavirus.

[a] Data are expressed as No. (%) of participants unless otherwise indicated. Data include all women evaluated in the 6-month and/or 12-month analyses, including 134 women evaluated in the 12-month analysis only. One woman from the HPV vaccine group was 26 years old at entry; 7 women (1 from HPV vaccine group and 6 from control group) had missing information on lifetime number of sexual partners; 8 women (4 from HPV vaccine group and 4 from control group) had missing cytologic results.

[b] Referral to colposcopy after trial entry based on evidence of low-grade squamous intraepithelial lesion or HPV-positive atypical squamous cells of uncertain significance at entry and at the 6-month visit or evidence of high-grade squamous intraepithelial lesion or more severe disease at the 6-month visit.

[c] Numbers in the HPV analysis groups add up to more than the total number of women because some women had multiple infections. Percentages were calculated at the infection level.

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group; $P=.91$). A total of 1671 women (825 in the HPV vaccine group and 846 in the control group; $P=.61$) had HPV PCR results available from the 12-month visit, including 134 women (56 in the HPV vaccine group and 78 in the control group) who did not have HPV PCR results available from the 6-month visit. Polymerase chain reaction results were not available for 705 women because of missed visits (n=456) or study discontinuations (n=65), because PCR results were not yet available from the testing laboratory (n=87), or because the 12-month study visit had not yet been conducted at the time of this analysis (n=97).

Of the 705 women excluded, 341 (48.4% vs 0 among those included in the analysis; $P<.001$) had missing 12-month data because of referral to colposcopy after study entry. Consequently, women excluded from the 12-month analysis differed from those included with respect to factors related to colposcopy referral, such as mean age (20.9 years vs 21.4 years; $P<.001$), mean lifetime number of sexual partners (2.9 vs 2.7; $P=.002$), frequency of abnormal cytologic findings at entry (62.8% vs 16.1%; $P<.001$), and proportion of individuals with multiple HPV infections (50.1% vs 40.2%; $P<.001$). However, these differences were nondifferential by study group; the 705 women were comparable by group with respect to age ($P=.22$), lifetime number of sexual partners ($P=.77$), abnormal cytologic findings at entry ($P=.93$), proportion of participants referred for colposcopy during follow-up ($P=.50$), proportion of individuals with multiple infections ($P=.24$), and distribution of HPV analysis groups ($P=.10$).

Different exclusions were applied for the analysis based on entry HC2 testing results because the intent of this specific analysis was to examine vaccine effects among the group of women positive for HPV by the clinically approved HC2 test. Of the 5758 women with pelvic evaluations who were not referred to colposcopy at entry, 3711 (1866 in the HPV vaccine group and 1845 in the control group) were excluded because they were HC2-negative, 181 (92 in the HPV vaccine group and 89 in the control group) were excluded because they were missing HC2 results from entry, and 104 (59 in the HPV vaccine group and 45 in the control group) were excluded because they were had positive HC2 results but negative PCR results.

Table 2. Viral Clearance and Vaccine Efficacy for Viral Clearance for HPV-16 and HPV-18 by Study Group at 6 Months and 12 Months of Follow-up

Follow-up Time, mo	No. Cleared/Total Infections (%)[a] HPV Vaccine Group	Control Group	Vaccine Efficacy for Viral Clearance, % (95% CI)
HPV-16			
6	47/172 (27.3)	61/222 (27.5)	–0.2 (–13.2 to 11.3)
12	54/123 (43.9)	73/159 (45.9)	–3.7 (–28.2 to 16.1)
HPV-18			
6	35/76 (46.1)	34/76 (44.7)	2.4 (–30.5 to 27.0)
12	32/54 (59.3)	37/61 (60.7)	–3.5 (–62.0 to 33.8)
HPV-16/18[b]			
6	82/248 (33.4)	95/298 (31.6)	2.5 (–9.8 to 13.5)
12	86/177 (48.8)	110/220 (49.8)	–2.0 (–24.3 to 16.3)
HPV-16/18 (restricted to women who received all vaccine doses)[c]			
6	81/241 (33.8)	93/288 (32.0)	2.6 (–10.1 to 13.8)
12	69/149 (46.5)	98/196 (50.0)	–7.0 (–31.7 to 13.0)
HPV-16/18 (restricted to women with single infections at entry)			
6	23/82 (28.0)	24/97 (24.7)	4.4 (–14.1 to 19.9)
12	28/63 (44.4)	37/79 (46.8)	–4.5 (–41.4 to 22.8)

Abbreviations: CI, confidence interval; HPV, human papillomavirus.
[a] Percentages calculated using generalized estimating equations method and may therefore vary from crude percentages.
[b] HPV-16/18 is defined as HPV-16 and/or HPV-18.
[c] All doses are defined as 2 doses at the 6-month follow-up and 3 doses at the 12-month follow-up.

A total of 1762 (874 in the HPV vaccine group and 888 in the control group) were positive for HPV DNA by both HC2 and PCR testing. The final group for this analysis consisted of 1520 of these 1762 women for whom HPV testing results were available from the 6-month visit (752 in the HPV vaccine group and 768 in the control group; $P=.68$). A total of 1169 of these women (574 in the HPV vaccine group and 595 in the control group; $P=.54$) also had HPV PCR results available from the 12-month follow-up, including 101 women (43 in the HPV vaccine group and 58 in the control group) who did not have HPV PCR results available from the 6-month visit. The data freeze dates for the various components of the trial data were as follows: clinical database, March 29, 2007; risk factor questionnaire, June 26, 2006; HPV PCR database, April 25, 2007; and HPV-16/18 serology database, July 17, 2006.

Characteristics of participants included in this analysis were compared between groups. Results are summarized in TABLE 1. The 1088 participants in the HPV vaccine group and 1101 participants in the control group were comparable with respect to age at entry, lifetime number of sexual partners, cytologic findings at entry, proportion of participants referred to colposcopic evaluation during follow-up, proportion of individuals with multiple infections, and distribution of HPV analysis groups. The number of doses received and intervals between entry and the 1-month, 6-month, and 12-month visits were also comparable across groups (Table 1).

HPV Viral Clearance

Rates of viral clearance at the 6-month and 12-month visits and VEVC estimates for HPV-16, HPV-18, and HPV-16/18 combined are presented in TABLE 2. At the 6-month visit, rates of

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clearance were 27.3% vs 27.5% for HPV-16, 46.1% vs 44.7% for HPV-18, and 33.4% vs 31.6% for HPV-16/18 among participants who received the HPV vaccine and the control vaccine, respectively. At the 12-month visit, rates of clearance among participants in the HPV group and the control group, respectively, were 43.9% vs 45.9% for HPV-16, 59.3% vs 60.7% for HPV-18, and 48.8% vs 49.8% for HPV-16/18.

There was no evidence that HPV vaccination significantly altered rates of viral clearance; VEVC estimates overall ranged from –0.2% to 2.5% at the 6-month visit and from –3.7% to –2.0% at the 12-month visit. For HPV-16/18 infections, the VEVC estimates were 2.5% (95% CI, –9.8% to 13.5%) at the 6-month visit and –2.0% (95% CI, –24.3% to 16.3%) at the 12-month visit. No significant evidence of a vaccine therapeutic effect was observed in analyses restricted to women who received all doses of vaccine or those with evidence of single HPV infections at entry (Table 2).

We observed no evidence of vaccine effects when we stratified the analysis on selected study entry characteristics reflective of disease extent, including HPV-16/18 antibody results, cytologic results, and HPV viral load (TABLE 3). Similarly, no evidence of vaccine effects was observed in analyses stratified by other study entry parameters thought to potentially influence clearance rates and efficacy of the vaccine, including time since sexual initiation, oral contraceptive use, cigarette smoking, and concomitant infection with *C trachomatis* or *N gonorrhoeae* (Table 3).

TABLE 4 presents results from analyses that evaluated rates of viral clearance and VEVC for HPV categories other than HPV-16 and HPV-18. Rates of viral clearance at the 6-month visit for these other HPV categories were higher than for HPV-16 in particular, ranging from 44.6% to 61.1% in the control group; clearance rates at the 12-

Table 3. Viral Clearance and Vaccine Efficacy for Viral Clearance for HPV-16/18 by Study Group at 6 Months and 12 Months of Follow-up, Stratified by Selected Factors[a]

	6-Month Follow-up			12-Month Follow-up		
	No. Cleared/Total Infections (%)[b]			No. Cleared/Total Infections (%)[b]		
Characteristics	HPV Vaccine Group	Control Group	Vaccine Efficacy for Viral Clearance, % (95% CI)	HPV Vaccine Group	Control Group	Vaccine Efficacy for Viral Clearance, % (95% CI)
Serologic findings at entry						
Unknown	6/31 (21.4)	15/39 (35.1)	–21.1 (–64.5 to 10.8)	9/21 (47.4)	14/28 (46.2)	2.3 (–70.4 to 43.9)
Negative	20/74 (26.9)	18/79 (22.7)	5.4 (–13.8 to 21.4)	22/49 (43.6)	26/57 (45.5)	–3.4 (–46.2 to 26.9)
Positive	56/143 (39.2)	62/180 (34.7)	6.9 (–10.5 to 21.6)	55/107 (51.4)	70/135 (52.3)	–1.7 (–32.7 to 22.0)
Cytologic findings at entry						
Unknown	0/3	0/1		0	0/1	
Normal	53/152 (35.0)	60/195 (30.6)	6.4 (–8.9 to 19.5)	69/147 (46.8)	95/188 (50.3)	–6.9 (–32.2 to 13.5)
Abnormal	29/93 (31.8)	35/102 (34.0)	–3.3 (–26.5 to 15.6)	17/30 (57.3)	15/31 (48.4)	17.2 (–41.9 to 51.7)
HC2 viral load at entry, relative light units						
Unknown	4/6 (66.7)	1/7 (14.3)	61.1 (–25.5 to 87.9)	3/4 (75.0)	2/5 (40.0)	58.3 (–162.9 to 93.4)
0-<2.0	13/41 (31.7)	19/54 (34.0)	–3.4 (–37.4 to 22.2)	18/37 (48.6)	28/52 (53.0)	–9.3 (–67.7 to 28.8)
2.0-<50	30/94 (32.0)	36/126 (28.6)	4.7 (–13.8 to 20.2)	34/80 (42.3)	52/107 (48.5)	–12.1 (–46.4 to 14.2)
≥50	35/107 (32.8)	39/111 (34.6)	–2.7 (–24.9 to 15.5)	31/56 (56.5)	28/56 (50.0)	13.1 (–30.4 to 42.0)
Time since sexual initiation, mo						
Unknown	0	0/1		0	0	
0-36	26/78 (35.1)	29/92 (30.2)	7.0 (–15.6 to 25.2)	26/52 (51.0)	30/65 (46.0)	9.2 (–30.3 to 36.7)
37-72	31/80 (38.3)	38/110 (35.0)	5.0 (–18.9 to 24.2)	33/61 (53.4)	43/82 (53.2)	0.6 (–42.8 to 30.8)
≥73	25/90 (27.8)	28/95 (29.0)	–1.7 (–22.1 to 15.3)	27/64 (42.7)	37/73 (49.4)	–13.2 (–54.8 to 17.3)
Oral or injectable contraceptive use						
Unknown	0	0/1		0	0	
No current use	31/101 (30.3)	34/118 (28.7)	2.3 (–16.5 to 18.1)	34/69 (48.7)	45/89 (51.0)	–4.6 (–43.4 to 23.8)
Current use	51/147 (35.3)	61/179 (33.8)	2.3 (–14.8 to 16.8)	52/108 (49.1)	65/131 (48.8)	0.5 (–28.2 to 22.8)
Smoking status						
Unknown	0	0/1		0	0	
Not current smoker	69/204 (34.1)	78/250 (31.1)	4.4 (–9.0 to 16.1)	78/151 (52.0)	90/184 (48.9)	6.2 (–17.1 to 24.8)
Current smoker	13/44 (29.3)	17/47 (34.9)	–8.6 (–45.8 to 19.1)	8/26 (30.8)	20/36 (54.4)	–51.9 (–136.7 to 2.5)
Chlamydia/gonorrhea status at entry						
Unknown	4/6 (66.7)	1/6 (16.7)	60.0 (–31.1 to 87.8)	3/4 (75.0)	1/4 (25.0)	66.7 (–99.5 to 94.4)
Negative	59/180 (33.5)	73/223 (32.5)	1.5 (–13.4 to 14.5)	66/127 (52.4)	84/160 (52.6)	–0.3 (–28.7 to 21.8)
Positive	19/62 (30.1)	21/69 (30.3)	–0.2 (–25.7 to 20.1)	17/46 (36.0)	25/56 (43.6)	–13.5 (–56.4 to 17.7)

Abbreviations: CI, confidence interval; HC2, Hybrid Capture 2 test; HPV, human papillomavirus.
[a] HPV-16/18 is defined as HPV-16 and/or HPV-18.
[b] Percentages calculated using generalized estimating equations method and may therefore vary from crude percentages.

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month visit ranged from 59.2% to 78.1%. There was no evidence that HPV vaccination significantly altered rates of viral clearance (VEVC estimates ranged from –7.6% to 7.6% at the 6-month visit and from –8.7% to 12.2% at the 12-month visit).

Table 4. Viral Clearance and Vaccine Efficacy for Viral Clearance for Other HPV Categories by Study Group at 6 Months and 12 Months of Follow-up

Viral Type	No. Cleared/Total Infections (%)[a]		Vaccine Efficacy for Viral Clearance, % (95% CI)
	HPV Group	Control Group	
6-mo Follow-up			
Species alpha-7 other than HPV-18	115/243 (47.0)	131/258 (50.8)	–7.6 (–27.9 to 9.5)
Species alpha-9 other than HPV-16	208/425 (48.8)	194/427 (44.6)	7.6 (–5.2 to 18.8)
Other oncogenic types	224/411 (54.5)	195/383 (50.9)	7.3 (–7.4 to 20.0)
Nononcogenic types	240/403 (59.4)	228/371 (61.1)	–4.5 (–24.6 to 12.4)
HC2-positive	649/1337 (48.0)	651/1372 (47.2)	1.5 (–6.3 to 8.8)
12-mo Follow-up			
Species alpha-7 other than HPV-18	119/178 (67.0)	133/209 (63.9)	8.4 (–21.0 to 30.7)
Species alpha-9 other than HPV-16	181/325 (55.7)	199/335 (59.2)	–8.7 (–30.1 to 9.1)
Other oncogenic types	236/322 (73.2)	216/299 (72.2)	3.5 (–25.2 to 25.7)
Nononcogenic types	264/327 (80.8)	254/326 (78.1)	12.2 (–18.3 to 34.8)
HC2-positive	624/977 (63.7)	660/1037 (63.4)	0.8 (–12.0 to 12.1)

Abbreviations: CI, confidence interval; HC2, Hybrid Capture 2 test; HPV, human papillomavirus.
[a]Percentages calculated using generalized estimating equations method and may therefore vary from crude percentages.

COMMENT

We evaluated the effect of HPV VLP-based vaccination on viral clearance among participants in our efficacy trial in Costa Rica with prevalent infection at the time of first vaccination. The trial has the advantage of being both community-based (participants were identified via a population census) and randomized. Our results show that rates of viral clearance over a 12-month period are not influenced by vaccination. Overall, VEVC estimates obtained were close to 0%, and the 95% CIs (eg, –24.3% to 16.3% for HPV-16/18 at 12 months) suggest that even if vaccination provides some benefit, it is likely to be small. The post hoc power estimates are 82%, 98%, and 99% for testing the hypothesis that the vaccine efficacy for HPV-16/18 clearance at 12 months is 30%, 40%, or 50%, respectively.

These findings have important clinical implications. For example, in countries where HPV DNA testing is incorporated in cervical cancer screening and prevention efforts,[19] adult women who have abnormal Papanicolaou test results induced by HPV infection and/or who test positive for an oncogenic HPV type using the clinically available HC2 test might be interested in receiving the HPV vaccine to treat their existent infection. Our results indicate that clinicians should discourage use of L1 VLP-based vaccines for this purpose.

Although VLP-based HPV vaccines are unlikely to provide benefit in the treatment of prevalent infections, women with established infections might benefit from vaccination in other ways at the individual level. From a population (or public health) perspective, however, it is unclear whether, among women infected with HPV, the residual benefit of preventing infection with HPV types contained in the vaccine to which the women have not yet been exposed would be sufficient to warrant vaccination. However, since HPVs are very common viruses to which women are typically exposed in the initial months or years following sexual debut,[1] vaccination of women after they have been exposed and infected (ie, after their peak period of exposure) is likely to be of less benefit than vaccination of women prior to initial exposure. This contrasts with other cervical cancer prevention approaches (Papanicolaou screening and HPV DNA testing), whose benefits are highest when women older than the typical peak of HPV acquisition and clearance (eg, ≥30 years) are targeted for evaluation.[27] Examination of the level of benefit provided by HPV vaccination of women who are past their peak period of exposure is currently under evaluation in several ongoing clinical efficacy trials.

It is also not clear whether vaccination would benefit women with prevalent infection by increasing their immunologic resistance against reappearance (via reactivation or reinfection) with the same viral type in the future. Because HPV-infected women who clear their infection have demonstrated immunocompetence to handle an established HPV infection, these women might be expected to be capable of handling subsequent infections without the need for vaccination. In particular, it is possible to hypothesize that T-cell responses developed following an initial HPV infection that successfully clears would be capable of adequately eradicating subsequent viral infections. This remains an open issue for future investigation.

In the present analysis, we avoided the evaluation of cytologic and/or histological data obtained after randomization as potentially compromising the stated primary objective of our randomized trial to evaluate the efficacy of the HPV-16/18 cervical cancer vaccine to protect women without HPV infection from the development of high-grade cervical precancers and cancer. We therefore cannot formally rule out the possibility that vaccination prevents progression to cytohistological outcomes. However, given that viral clearance rates did not differ by treatment group and that persistent viral infection is the best established predictor of risk of progression, it is unlikely that vaccination could have a significant beneficial impact on rate of lesion progression.[1,17]

Since the trial is ongoing and investigators and site personnel have no access to safety data by treatment group, we also did not evaluate the safety profile of the vaccine in this analysis. How-

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ever, efficacy results from the present analysis caution against the benefit of vaccination to treat established infections, regardless of safety profile. Given published data suggesting the overall safety of the present vaccine,[3,28] a main residual question of interest is whether reactogenicity and adverse event profiles differ by HPV infection status at the time of vaccination. Data to evaluate these questions directly will be available after unblinding occurs in our and other ongoing trials.

We evaluated viral clearance at 6 and 12 months after enrollment. While the evaluation of viral clearance at 6 months had the advantage of including more than 85% of participants with prevalent HPV infection at enrollment, it evaluated efficacy before the full series of vaccinations were administered. Conversely, the evaluation of viral clearance at 12 months had the advantage of evaluating clearance rates after the full series of vaccinations were administered but included a smaller proportion (70%) of participants with prevalent HPV infection at enrollment. It is reassuring to note that results were consistent in both approaches.

Results from our community-based study provide strong evidence that there is little, if any, therapeutic benefit from the vaccine in the population we studied. Furthermore, we see no reason to believe that there is therapeutic benefit of the vaccine elsewhere because the biological effect of vaccination among already infected women is not expected to vary by population. We should note that while the vaccine efficacy estimates provided herein are accurate, the absolute clearance estimates reported within individual study groups may be too high because we excluded women referred to colposcopy from the analysis; infections in these women are undoubtedly less likely to clear than in women who were not referred.

In summary, our results demonstrate that in women positive for HPV DNA, HPV-16/18 vaccination does not accelerate clearance of the virus and should not be used for purposes of treating prevalent infections.

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Obtained funding: Hildesheim, Herrero, Dubin, Lowy.
Administrative, technical, or material support: Herrero, Solomon, Bratti, Gonzalez, Porras, Jimenez
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Financial Disclosures: Drs Schiller and Lowy report that they are named inventors on US government–owned HPV vaccine patents that are licensed to GSK and Merck and are entitled to limited royalties as specified by federal law. Dr Dubin is employed by GSK Biologicals, the manufacturer of the vaccine used in this trial. No other financial disclosures were reported.

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Funding/Support: The Costa Rican HPV Vaccine Trial is a long-standing collaboration between investigators in Costa Rica and the NCI. The trial is sponsored and funded by the NCI (grant N01-CP-11005), with funding support from the National Institutes of Health Office for Research on Women's Health, and conducted with support from the Ministry of Health of Costa Rica. Vaccine was provided for our trial by GSK Biologicals, under a Clinical Trials Agreement with the NCI. GlaxoSmithKline also provided support for aspects of the trial associated with regulatory submission needs of the company under grant FDA BB-IND 7920.

Role of the Sponsors: The NCI and Costa Rica investigators are responsible for the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation of the manuscript. The NCI and Costa Rica investigators make final editorial decisions on this and subsequent publications; GSK has the right to review and comment.

Additional Contributions: We thank the women of Guanacaste and Puntarenas, Costa Rica, who participated in this study. We also acknowledge the effort and dedication of the staff in Costa Rica involved in this project, including Bernardo Blanco and his team (census); Ricardo Cerdas and Ana Hernández (blood processing); Osman López, Johnny Matamoros, Cristian Montero, Rafael Thompson, and Jorge Umaña (field activity coordinators); Su Yen Araya, Hazel Barquero, Hayleen Campos, Muriel Grijalba, Ana Cristina Monge, Ana Peraza, Diana Robles, María Fernanda Sáenz, Dorita Vargas, and Jessica Vindas (clinic coordinators); Paola Alvarez, Dinia Angulo, Ana Live Arias, Betzaida Barrantes, Marianela Bonilla, Jessenia Chinchilla, Marianela Herrera, Andrea Interiano, Viviana Loría, Rebeca Ocampo, Angie Ramírez, Libia Rivas, Jessenia Ruiz, Malena Salas, and Yesenia Vázquez (clinicians); Marta Alvarado, Ana Cristina Arroyo, Gloriana Barrientos, Diana Díaz, Marlen Jara, Maureen Matarrita, María Ester Molina, Elida Ordóñez, Gina Sánchez, and Siara Villegas (nurses); Arianne Castrillo and Vivian Lopez (education and outreach effort coordinators); Karla Coronado (appointment coordinator); Ricardo Alfaro (quality control coordinator); Charles Sanchez and Livia Romero (document center coordinators); and Eric Alpizar and Carlos Avila (information technology coordinators). We especially recognize Sofia Elizondo, executive director of Fundación INCIENSA, and her staff for administrative support. We appreciate the team from Information Management Services, Silver Spring, Maryland, responsible for the development and maintenance of the data system used in the trial and who serve as the data management center for this effort, specifically the contributions of Julie Buckland, Jean Cyr, Laurie Rich, and John Schussler. We acknowledge the contributions made by individuals at Westat Inc, Rockville, Maryland, who provided project development and/or monitoring support, including Maribel Gomez, Kirk Midkiff, Kerrygrace Morrisey, and Susan Truitt. We acknowledge the assistance provided by Carla Chorley, Troy Moore, Kathi Shea, and Heather Siefers in the establishment of a specimen and vaccine repository for our trial and in their continued assistance with the handling and shipment of specimens. From GSK Biologicals, we acknowledge Anne Schuind, Kelechi Lawrence, Darrick Fu, and Bruce Innis for their contribution to discussions regarding trial conduct and Francis Dessy and Brigitte Colau for HPV-16/18 antibody testing.

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