

## ARTICLE

# Multisite HPV16/18 Vaccine Efficacy Against Cervical, Anal, and Oral HPV Infection

Daniel C. Beachler, Aimée R. Kreimer, Mark Schiffman, Rolando Herrero, Sholom Wacholder, Ana Cecilia Rodriguez, Douglas R. Lowy, Carolina Porras, John T. Schiller, Wim Quint, Silvia Jimenez, Mahboobeh Safaeian, Linda Struijk, John Schussler, Allan Hildesheim, Paula Gonzalez; for the Costa Rica HPV Vaccine Trial (CVT) Group

**Affiliations of authors:** Division of Cancer Epidemiology, and Genetics (DCB, ARK, MS, SW, MS, AH) and Center for Cancer Research (DRL, JTS), National Cancer Institute, NIH, Bethesda, MD; Proyecto Epidemiológico Guanacaste, Fundación INCIENSA, Costa Rica (RH, ACR, CP, SJ, PG); Early Detection and Prevention Section, International Agency for Research on Cancer, Lyon, France (RH); DDL Diagnostic Laboratory, Voorburg, the Netherlands (WQ, LS); Information Management Systems, Rockville, MD (JS).

**Correspondence to:** Daniel C. Beachler, PhD, MHS, 9609 Medical Center Drive, Room 6E-220, Bethesda, MD 20892 (e-mail: [daniel.beachler@nih.gov](mailto:daniel.beachler@nih.gov)).

## Abstract

**Background:** Previous Costa Rica Vaccine Trial (CVT) reports separately demonstrated vaccine efficacy against HPV16 and HPV18 (HPV16/18) infections at the cervical, anal, and oral regions; however, the combined overall multisite efficacy (protection at all three sites) and vaccine efficacy among women infected with HPV16 or HPV18 prior to vaccination are less known.

**Methods:** Women age 18 to 25 years from the CVT were randomly assigned to the HPV16/18 vaccine (Cervarix) or a hepatitis A vaccine. Cervical, oral, and anal specimens were collected at the four-year follow-up visit from 4186 women. Multisite and single-site vaccine efficacies (VEs) and 95% confidence intervals (CIs) were computed for one-time detection of point prevalent HPV16/18 in the cervical, anal, and oral regions four years after vaccination. All statistical tests were two-sided.

**Results:** The multisite woman-level vaccine efficacy was highest among “naïve” women (HPV16/18 seronegative and cervical HPV high-risk DNA negative at vaccination) (vaccine efficacy = 83.5%, 95% CI = 72.1% to 90.8%). Multisite woman-level vaccine efficacy was also demonstrated among women with evidence of a pre-enrollment HPV16 or HPV18 infection (seropositive for HPV16 and/or HPV18 but cervical HPV16/18 DNA negative at vaccination) (vaccine efficacy = 57.8%, 95% CI = 34.4% to 73.4%), but not in those with cervical HPV16 and/or HPV18 DNA at vaccination (anal/oral HPV16/18 VE = 25.3%, 95% CI = -40.4% to 61.1%). Concordant HPV16/18 infections at two or three sites were also less common in HPV16/18-infected women in the HPV vaccine vs control arm (7.4% vs 30.4%,  $P < .001$ ).

**Conclusions:** This study found high multisite vaccine efficacy among “naïve” women and also suggests the vaccine may provide protection against HPV16/18 infections at one or more anatomic sites among some women infected with these types prior to HPV16/18 vaccination.

Consistent with other randomized trials (1,2), the National Cancer Institute (NCI)-sponsored, community-based human papillomavirus (HPV) 16/18 Costa Rica Vaccine Trial (CVT) demonstrated strong prophylactic vaccine efficacy against persistent

cervical HPV16 and HPV18 (HPV16/18) infection (3) and cervical intraepithelial neoplasia 2 or more severe disease (CIN2+) associated with those types (4). While HPV vaccination has the potential to substantially reduce the cervical cancer burden,

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several countries have documented recent increases in HPV-associated anal and oropharyngeal cancer (5,6). In fact, these HPV-associated noncervical cancers now account for over half of the HPV-associated cancer burden in the United States (5,7).

Less is known about the vaccine efficacy (VE) at noncervical sites in women, as the vaccines were licensed on the basis of cervical clinical outcomes (CIN2+). The quadrivalent HPV vaccine subsequently received an indication for prevention of anal cancer after efficacy was demonstrated against anal intraepithelial neoplasia in men (8). However, the vaccines do not have an indication for prevention of oropharyngeal cancer in men or women, partially because of the lack of a detectable precancerous lesion (9). However, more recent reports have focused on virologic endpoints (10), given that HPV infection is the etiologic agent in these cancers. Indeed, recent reports from the CVT have suggested that the VE against a single measurement of anal and oral HPV16/18 is high and similar to that against cervical HPV16/18 (11,12).

While the HPV16/18 vaccine appears efficacious at multiple sites in women “naïve” to HPV16/18 infection, the single-site VEs and combined multisite VE (protection at all three: cervical, anal, and oral) sites are less understood among women with prior infection. Previous reports suggest the HPV16/18 vaccine has no therapeutic effect on current cervical HPV16/18 infection (13), but it is possible that the vaccine may prevent HPV16/18 infection in at-risk (noninfected) anatomical sites and/or reduce reinfection in women previously or currently exposed to HPV16/18 at the time of vaccination. Thus, we examined the combined multisite and single-site VEs in sexually active women, including those who were never, formerly, or currently exposed to HPV16 or HPV18 infection at the time of vaccination.

## Methods

### Study Design and Laboratory Procedures

In the CVT, women age 18 to 25 years were randomly assigned at enrollment from 2004 to 2005 to be vaccinated with the bivalent HPV16/18 vaccine (Cervarix, GlaxoSmithKline Biologicals, Rixensart, Belgium) or a Hepatitis A vaccine (modified Havrix, GSK Biologicals) (3,14). This trial included three-dose vaccination (provided over six months) and active annual follow-up. At the enrollment and follow-up visits, risk-factor questionnaires were administered and a pelvic examination was performed on sexually active women. Exfoliated cervical cells were collected in PreservCyt medium (Cytoc Corp, now Hologic, Marlborough, MA) for liquid-based cytology and HPV DNA testing. In addition, blood was collected at enrollment to evaluate HPV16/18 serologic status. Further design and methods for the CVT have been previously described (3,14). The institutional review boards of the NCI and the Costa Rican Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA) approved this trial, and all study participants signed institutional review board-approved consent forms giving written informed consent. The trial is registered at clinicaltrials.gov, identifier NCT00128661.

Anal and oral specimens were also collected at the four-year visit, the final blinded visit of the CVT. The anal specimen was collected prior to the pelvic exam among sexually active women (defined by a history of vaginal intercourse) by inserting a dry swab 3 to 4 centimeters into the anal canal, rotating it one time, and then removing the swab while rotation continued using pressure against the wall of the anal canal. The swab was placed in 1 mL of PreservCyt and frozen immediately in liquid nitrogen. The oral rinse specimen was collected by use of a 30-second oral

rinse and gargle (15-second rinse/15-second gargle) with 15 mL of Scope mouthwash (Procter and Gamble Company, Cincinnati, OH) and was kept between 2° and 8° Celsius until same-day processing at the local laboratory (15,16).

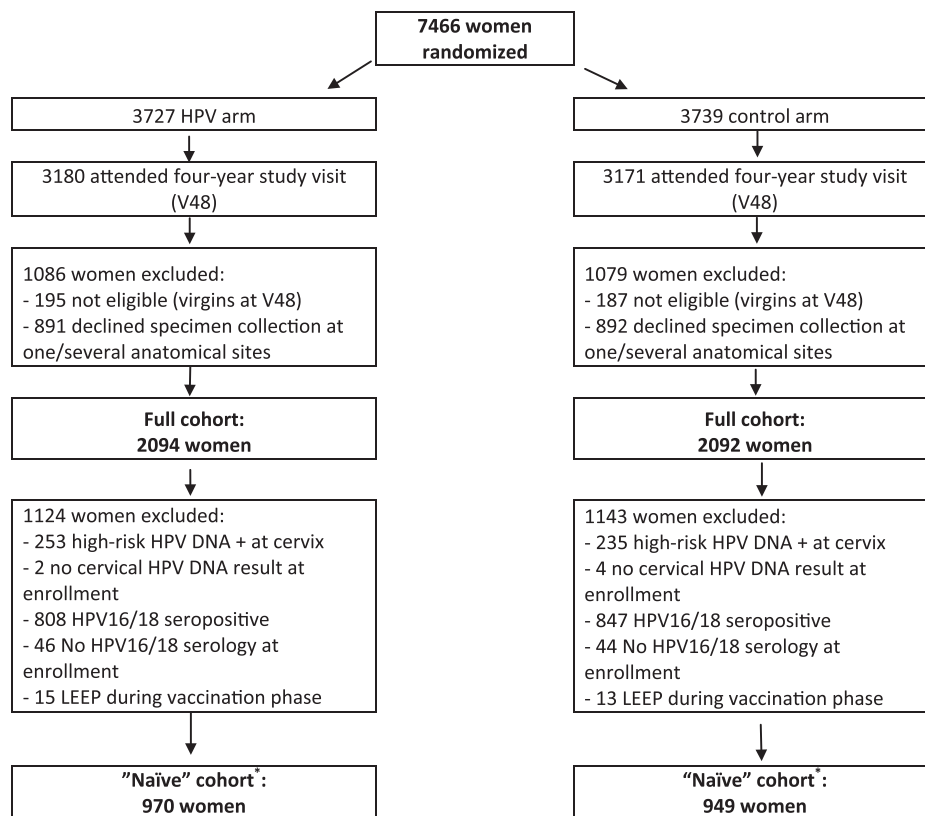
DNA was extracted from the cervical, oral, and anal samples through the MagNAPure LC DNA isolation procedure (Roche Diagnostics, Indianapolis, IN), and the extracted DNA was used for each polymerase chain reaction. Specimens were then tested for HPV DNA types utilizing the SPF<sub>10</sub> PCR-DEIA (DNA enzyme immunoassay)-LiPA<sub>25</sub> version 1 method (Labo Biomedical Products, Rijswijk, the Netherlands) (17,18). The LiPA<sub>25</sub> system detects 25 HPV genotypes, including 12 high-risk (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) types. To increase the sensitivity of HPV16/18 detection, all positive specimens on SPF<sub>10</sub> PCR/DEIA that were negative for HPV16 or HPV18 by LiPA<sub>25</sub> were also tested using HPV16 and 18 type-specific primers (19).

Baseline HPV16 and HPV18 serological status was determined using a virus-like particle-based direct enzyme-linked immunosorbent assay (VLP-ELISA), a standard assay that measures polyclonal antibodies (GlaxoSmithKline Biologicals) (20,21). Serologic results were dichotomized utilizing standard cutoffs calculated as antibody titer values three standard deviations above the geometric mean titers obtained from HPV-negative self-reported virgins (20). Individuals with an optical density of at least 8 ELISA units/mL for anti-HPV16 and at least 7 ELISA units/mL for anti-HPV18 were considered seropositive (20–22).

### Statistical Analyses

The full study population included all women who consented to cervical, anal, and oral samples at the four-year CVT visit and had HPV DNA test results available (Figure 1). Previous CVT reports have compared the characteristics of women who accepted or declined the oral rinse and/or anal swab samples and have demonstrated that the acceptance was similar across arms but higher among women reporting more lifetime sex partners and those reporting anal and oral sex (11,12). There were no oral/anal specimens collected prior to the four-year follow-up visit, which would have allowed for a more restricted naïve population with exclusion of women with prevalent oral or anal HPV16 or HPV18 DNA at enrollment. Given the age ranges of this study population (age 18–25 years at time of vaccination), we divided women into three distinct categories based on their HPV16/18 status at the time of vaccination. These categories included: 1) the “naïve” cohort that had no evidence of prior infection (negative for cervical high-risk HPV DNA and HPV16/18 antibodies at enrollment and did not receive cervical excision treatment [loop electrosurgical excision procedure {LEEP}] during the vaccination phase), 2) evidence of previous exposure (serologically HPV16 or HPV18 positive but cervical HPV16/18 DNA-negative), and 3) currently exposed (cervical HPV16 or HPV18 DNA positive regardless of serologic status).

VEs and 95% confidence intervals (CIs) were computed for one-time detection of HPV in the cervical, anal, and oral regions in full and subpopulations. VE represents the percent change (reduction or increase) in outcome observed when the HPV arm is compared against the control arm. We calculated the combined multisite woman-level VE, where an event (among  $n = 4186$  women) was defined as a woman with HPV16 and/or HPV18 DNA at any of the cervical, anal, or oral regions at the four-year visit. We also calculated combined multisite infection level analyses, which can be considered as weighted averages of the cervical, anal, and oral HPV16/18 VE results. An event in the multisite infection-level VE analysis ( $n = 12\,558$  sites)



**Figure 1.** Consort diagram for this Costa Rica Vaccine trial-based study. Asterisks indicate no baseline anal or oral sampling, so a true “According-to-Protocol” analysis could not be included. HPV = human papillomavirus; LEEP = loop electrosurgical excision procedure.

was defined as a woman’s anatomical site having HPV16 and/or HPV18 DNA (ie, each woman contributed three times). The VEs for the infection-level analysis were calculated utilizing the generalized estimating equation (GEE) method to adjust for the correlation within an individual (23). Exact confidence intervals for single-site VEs were calculated based on the binomial distribution of the number of events, while the multisite VEs that included GEE methods utilized mid-p corrected exact confidence limits because of their incompatibility with binomial conditioning. A concordant HPV infection was defined as a type-specific infection being present at two or three anatomic sites examined in this study. HPV concordance between different anatomic sites in the vaccine and control arms was compared while examining whether the vaccine may protect at least one or two of the three anatomic sites among women who may have been previously exposed to HPV16/18 infection. Type-specific concordance was also evaluated in pair-wise comparisons of sites within women using Kappa statistics with 95% confidence intervals. Chi-square tests were utilized to calculate *P* values. A *P* value of less than .05 was considered statistically significant, and all statistical tests were two-sided.

## Results

Among the 7466 women randomly assigned to the two arms in the CVT, 6351 (85.1%) attended the four-year study visit (Figure 1). Among these women, 2165 were excluded from the full cohort because they were virgins at the four-year follow-up visit (*n* = 382) or declined an anal, oral, or cervical specimen collection at this visit (*n* = 1783). The full analytical cohort therefore included 4186 women (2094 in the HPV vaccine arm and 2092

in the control arm). There were 1919 women in the restricted (naïve) cohort (970 in the HPV arm, 949 in the control arm), who were HPV16/18 seronegative at enrollment, high-risk HPV DNA-negative at enrollment, and did not receive LEEP therapy during the vaccination phase. Overall, 52.9% of the study population was both seronegative and cervical DNA negative for HPV16/18 infection at the time of vaccination, while 35.9% of participants were HPV16 or HPV18 seropositive but cervical HPV16/18 DNA negative, and 11.2% had a current cervical HPV16 or HPV18 infection (DNA) at vaccination.

The distributions of prerandomization characteristics were similar in the HPV and control arms, including age, cervical cytology, and cervical low-risk and high-risk HPV DNA positivity at enrollment (*P* > .05) (Supplementary Table 1, available online). In addition, postrandomization characteristics among the women were similar between the HPV and control arms, including self-reported oral, vaginal, and anal sex at the four-year follow-up visit. The rate of acceptance to provide an anal and an oral sample was also similar across arms (Figure 1).

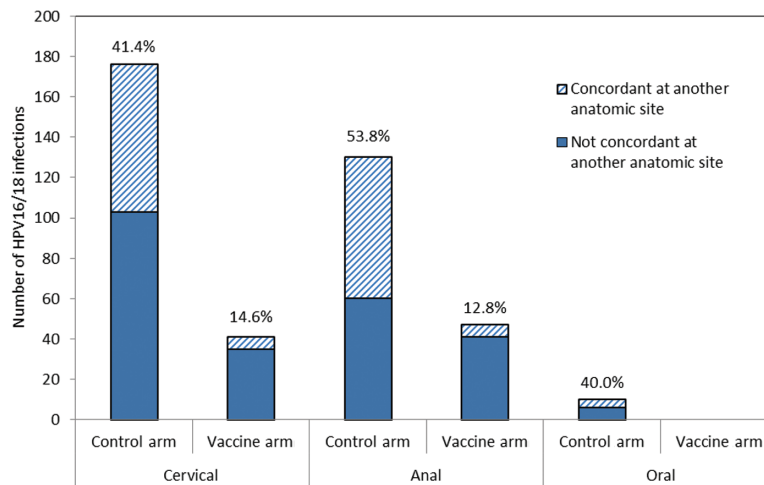
In the full cohort, the combined multisite woman-level HPV16/18 vaccine efficacy (VE) at the four-year follow-up visit was 64.8% (95% CI = 54.8% to 72.8%) (Table 1). Among the 2094 women in the vaccine arm, 81 had an HPV16 or HPV18 infection at one or more anatomic sites (3.9%), while 230 of the 2092 women in the control arm (11.0%) had an HPV16 or HPV18 infection at one or more anatomic sites (*P* < .001). While multisite woman-level HPV16/18 VE (for infection at one or more site) was evident in this population, it was higher for the combined multisite infection-level HPV16/18 VE of 71.2% (95% CI = 62.8% to 77.7%), although the difference was not statistically significant (Table 1). The difference between these two multisite measures

**Table 1.** HPV16/18 Vaccine efficacy against prevalent cervical, anal, and oral HPV16/18 DNA four years after vaccination in the full cohort

Anatomic site	Arm	Women or sites	Events	HPV16/18 prevalence, %	Efficacy (95% CI), %
Cervical, anal, or oral HPV16/18 multisite woman-level*	HPV vaccine	2094	81	3.9	64.8 (54.8 to 72.8)
	Control	2092	230	11.0	
Cervical HPV16/18	HPV vaccine	2094	40	1.9	76.4 (66.9 to 83.4)
	Control	2092	169	8.1	
Anal HPV16/18	HPV vaccine	2094	47	2.2	62.1 (47.3 to 73.1)
	Control	2092	124	5.9	
Oral HPV16/18	HPV vaccine	2094	0	0.0	100.0 (60.5 to 100.0)
	Control	2092	9	0.4	
Cervical, anal, oral HPV16/18 multisite infection level†	HPV vaccine	6282	87	1.4	71.2 (62.8 to 77.7)
	Control	6276	302	4.8	
HPV16/18 at two or three sites	HPV vaccine	2094	6	0.3	91.4 (81.4 to 96.6)
	Control	2092	70	3.3	

\* A woman-level event is defined as a woman who is positive for cervical, anal, and/or oral human papillomavirus DNA at the four-year visit. CI = confidence interval; HPV = human papillomavirus; VE = vaccine efficacy.

† An infection-level event is defined as a woman's anatomical site having HPV16/18 DNA (women contribute three times for each anatomic site); the VEs for this analysis utilized generalized estimating equations to adjust for the correlation within an individual.



**Figure 2.** Type-specific concordance of human papillomavirus (HPV) 16/18 infections at the four-year follow-up visit among 2092 women in the control arm and 2094 women in the HPV vaccine arm. The kappa for anal/cervical HPV16/18 agreement was 0.44 (95% confidence interval [CI] = 0.37 to 0.51) for the control arm and 0.13 (95% CI = 0.03 to 0.23) for the vaccine arm. The percentages on the figure indicate the amount of HPV16/18 infections that were concordant at another anatomic site. Seventy-five of the 79 concordant infections were anal/cervical infections. One individual was infected at all three sites. HPV = human papillomavirus.

was accounted for by a difference in HPV concordance (ie, type-specific HPV16/18 at two or three anatomic sites) in the two arms. Seventy of the 230 (30.4%) HPV16/18-infected women in the control arm had the same type-specific HPV16/18 infection at two or three anatomic sites, compared with only six of the 81 (7.4%) HPV16/18-infected women in the HPV vaccine arm ( $P < .001$ ). Indeed, the VE against type-specific concordant HPV16/18 infection at two or three anatomic sites was statistically significantly higher (VE = 91.4%, 95% CI = 81.4% to 96.6%) (Table 1) than the multisite woman-level estimates. HPV concordance was most often found between the cervix and anus (75 of 79 concordant infections), and the percent agreement between cervical and anal HPV16/18 was considerably lower in the HPV vaccine arm than the control arm (kappa = 0.13 vs 0.44,  $P < .001$ ) (Figure 2).

Compared with the HPV16/18 VE demonstrated in the full cohort, the combined multisite woman-level HPV16/18 VE was higher (VE = 83.5%, 95% CI = 72.1% to 90.8%) (Table 2) among the 1919 women in the “naïve” cohort representing women less likely to be exposed to HPV16/18 prior to vaccination. Similar to the full

cohort, there was a higher multisite infection-level VE (VE = 100.0%, 95% CI = 91.0% to 100.0%) (Table 2) than multisite woman-level VE because of a high VE against HPV16/18 concordance.

While HPV16/18 VE was highest in the naïve cohort, there was evidence of VE against cervical (VE = 76.5%, 95% CI = 54.6% to 88.8%) and anal HPV16/18 (VE = 54.4%, 95% CI = 22.4% to 73.9%) in the 1384 women with evidence of HPV16/18 infection prior to vaccination (HPV16 and/or HPV18 seropositive, but cervical HPV16/18 DNA negative at enrollment) (Table 3). Additionally, there was evidence of vaccine efficacy against the composite outcome of infection at the cervical, anal, or oral sites (multisite woman-level HPV16/18 VE = 57.8%, 95% CI = 34.4% to 73.4%) and even higher efficacy against HPV16/18 at two or three sites (VE = 90.6%, 95% CI = 65.8% to 98.5%) (Table 3). The multisite VE was not limited to the HPV type women were never exposed to, as there was evidence of multisite VE against HPV16 specifically among women who were HPV16 seropositive and cervical HPV16 DNA negative at baseline (VE = 47.7%, 95% CI = 3.2% to 72.7%) (Table 4; Supplementary Table 3, available online) and

**Table 2.** HPV16/18 vaccine efficacy against prevalent cervical, anal, and oral HPV16/18 DNA four years after vaccination in the “Naïve” cohort\*,†

Study population	Arm	Women or sites	No. events	HPV16/18 prevalence, %	Efficacy (95% CI), %
Cervical, anal, or oral HPV16/18	HPV vaccine	970	15	1.5	83.5 (72.1 to 90.8)
Multisite woman level‡	Control	949	89	9.4	
Cervical HPV16/18	HPV vaccine	970	8	0.8	89.4 (79.0 to 95.2)
	Control	949	74	7.8	
Anal HPV16/18	HPV vaccine	970	7	0.7	85.1 (68.4 to 93.8)
	Control	949	46	4.8	
Cervical, anal, oral HPV16/18	HPV vaccine	2910	15	0.5	88.2 (79.6 to 93.1)
Multisite infection level§	Control	2847	124	4.4	
HPV16/18 at two or three sites	HPV vaccine	970	0	0.0	100.0 (91.0 to 100.0)
	Control	949	34	3.6	

\* The naïve cohort excluded those who were human papillomavirus (HPV) 16/18 seropositive at baseline or high-risk cervical HPV DNA+ at baseline or had loop electrosurgical excision procedure during the vaccination phase. CI = confidence interval; HPV = human papillomavirus.

† The vaccine efficacy for oral HPV16/18 alone was excluded given low number of events. These results are presented in [Supplementary Table 2](#) (available online).

‡ A woman-level event is defined as a woman who is positive for cervical, anal, and/or oral HPV DNA at the four-year visit.

§ An infection-level event is defined as a woman's anatomical site having HPV16/18 DNA (women contribute three times for each anatomic site); the vaccine efficacies for this analysis utilized generalized estimating equations to adjust for the correlation within an individual.

**Table 3.** Multisite HPV16/18 vaccine efficacy among individuals with evidence of a previous\* HPV16 or HPV18 infection prior to vaccination†

Study population	Arm	Women or sites	No. events	HPV16/18 prevalence, %	Efficacy (95% CI), %
Cervical, anal, or oral HPV16/18	HPV vaccine	681	27	4.0	57.8 (34.4 to 73.4)
Multisite woman level‡	Control	703	66	9.4	
Cervical HPV16/18	HPV vaccine	681	10	1.5	76.5 (54.6 to 88.8)
	Control	703	44	6.3	
Anal HPV16/18	HPV vaccine	681	19	2.8	54.4 (22.4 to 73.9)
	Control	703	43	6.1	
Cervical, anal, oral HPV16/18	HPV vaccine	2043	29	1.4	66.4 (47.0 to 78.6)
Multisite infection level§	Control	2109	89	4.2	
HPV16/18 at two or three sites	HPV vaccine	681	2	0.3	90.6 (65.8 to 98.5)
	Control	703	22	3.1	

\* Defined as women who were human papillomavirus (HPV) 16 and/or HPV18 seropositive at baseline and cervical HPV16/18 DNA negative at baseline.

CI = confidence interval; HPV = human papillomavirus.

† The vaccine efficacy for oral HPV16/18 alone was excluded given low number of events. These results are presented in [Supplementary Table 2](#) (available online).

‡ A woman-level event is defined as a woman who is positive for cervical, anal, and/or oral HPV DNA at the four-year visit.

§ An infection-level event is defined as a woman's anatomical site having HPV16/18 DNA (women contribute three times for each anatomic site); the vaccine efficacies for this analysis utilized generalized estimating equations to adjust for the correlation within an individual.

|| Statistically significantly lower HPV16/18 concordance at two or more anatomic sites in HPV16/18-infected women in the HPV vaccine arm (7.4% [2/27] of HPV16/18-infected women) than the control arm (33.3% [22/66] of HPV16/18-infected women),  $P = .01$ .

VE against HPV18 among those who were HPV18 seropositive and cervical HPV18 DNA negative at baseline (VE = 47.8%, 95% CI = -22.3% to 79.0%) ([Table 4](#); [Supplementary Table 3](#), available online). However, this VE was restricted to women with lower enrollment antibody titers, as there was no statistically significant multisite or cervical VE in those with a titer higher than the median titer (32 EU/mL for HPV16, 17 EU/mL for HPV18) ([Table 4](#)).

In contrast to the protection seen in women exposed to HPV16 or HPV18 prior to vaccination, there was no statistically significant evidence of HPV16/18 VE in the 407 women with a current cervical HPV16/18 infection at the time of vaccination against anal/oral HPV16/18 (VE = 25.3%, 95% CI = -40.4% to 61.1%) ([Table 5](#)) or against cervical HPV16/18 re-infection ([Table 5](#); [Supplementary Table 4](#), available online).

## Discussion

This randomized trial demonstrated multisite vaccine efficacy against cervical, anal, and oral HPV16/18 at the four-year follow-up visit among women with no evidence of HPV16 or HPV18

exposure and among some women who may have cleared/controlled their HPV16/18 infection prior to vaccination. In addition, HPV16/18-infected women in the vaccine arm were less likely to have an HPV16 or an HPV18 infection at two or more anatomic sites than HPV16/18-infected women in the control arm. These findings suggest that while the HPV16/18 vaccine is most efficacious among women prior to sexual debut, the vaccine may provide protection against HPV at some or all at-risk anatomic sites in many sexually active women.

The lower but statistically significant multisite VE in formerly HPV16/18-infected women is important given their abundance in sexually active populations, as they represented over one-third of this study population. The multisite vaccine efficacy among women exposed to HPV16/18 prior to vaccination reinforces findings from other HPV vaccine trials, which suggested that there was some VE against cervical HPV 16/18 among women who were HPV16/ or HPV 18 seropositive and cervical HPV 16/18 DNA negative at the time of vaccination ([3,24,25](#)). This study finds that these previously HPV16/18-exposed women may be protected not only at the cervix, but also at the anal

**Table 4.** HPV16 and HPV18 vaccine efficacies by antibody titer among individuals with evidence of a previous\* HPV16 or HPV18 infection prior to vaccination

Population	Arm	No. women	No. events*	HPV16/18 prev., %	Efficacy (95% CI), %
HPV16 seropositive and cervical HPV16 DNA negative at baseline					
Cervical, anal, or oral HPV16	HPV vaccine	477	15	3.1	47.7 (3.2 to 72.7)
Multisite woman level†	Control	482	29	6.0	
Cervical HPV16	HPV vaccine	477	5	1.0	71.9 (27.4 to 90.7)
	Control	482	18	3.7	
HPV16 titer below the median titer (<32 EU/mL)					
Cervical, anal, or oral HPV16	HPV vaccine	247	4	1.6	73.4 (25.3 to 92.3)
Multisite woman level‡	Control	279	17	6.1	
Cervical HPV16	HPV vaccine	247	3	1.2	75.8 (21.9 to 94.4)
	Control	279	14	5.0	
HPV16 titer at or above the median titer (≥32 EU/mL)					
Cervical, anal, or oral HPV16	HPV vaccine	230	11	4.8	19.1 (-86.5 to 65.2)
Multisite woman level‡	Control	203	12	5.9	
Cervical HPV16	HPV vaccine	230	2	0.9	55.9 (-148.8 to 94.3)
	Control	203	4	2.0§	
HPV18 seropositive and cervical HPV18 DNA negative at baseline					
Cervical, anal, or oral HPV18	HPV vaccine	509	8	1.6	47.8 (-22.3 to 79.0)
Multisite woman level‡	Control	498	15	3.0	
Cervical HPV18	HPV vaccine	509	2	0.4	78.3 (8.7 to 96.8)
	Control	498	9	1.8	
HPV18 titer below the median titer (<17 EU/mL)					
Cervical, anal, or oral HPV18	HPV vaccine	249	2	0.8	81.0 (23.7 to 97.1)
Multisite woman level‡	Control	260	11	4.2	
Cervical HPV18	HPV vaccine	249	0	0.0	100.0 (52.6 to 100.0)
	Control	260	8	3.1	
HPV18 titer at or above the median titer (≥17 EU/mL)					
Cervical, anal, or oral HPV18	HPV vaccine	260	6	2.3	-37.3 (-451.8 to 62.4)
Multisite woman level‡	Control	238	4	1.7	
Cervical HPV18	HPV vaccine	260	2	0.8	-83.1 (-5300.3 to 86.1)
	Control	238	1	0.4	

\* Defined as women who were human papillomavirus (HPV) 16 seropositive and cervical HPV16 DNA negative at baseline for the HPV16 analysis, and HPV18 seropositive and cervical HPV18 DNA negative at baseline for the HPV18 analysis. CI = confidence interval; HPV = human papillomavirus.

† For this analysis, events were defined as detection of type-specific HPV16 or HPV18 DNA at the four-year visit among women who were HPV16 or HPV18 seropositive for that same HPV type at baseline (ie, the analysis was restricted to examining the potential protection against the HPV type that the women previously had prior to vaccination).

‡ A woman-level event is defined as a woman who is positive for cervical, anal, and/or oral HPV DNA at the four-year visit.

§ Cervical HPV16 prevalence at the four-year visit among high-titer HPV16 seropositive/HPV16 DNA-negative individuals was not statistically significantly lower than the cervical HPV16 prevalence in the control arm of the naïve cohort (2.0% vs 5.1%,  $P = .06$ ).

|| Cervical HPV18 prevalence at the four-year visit among high-titer HPV18 seropositive/HPV18 DNA-negative individuals was statistically significantly lower than the cervical HPV18 prevalence in the control arm of the naïve cohort (0.4% vs 3.2%,  $P = .02$ ).

**Table 5.** HPV16/18 vaccine efficacy among individuals with cervical HPV16 or HPV18 DNA detected at enrollment

Study population	Arm	Women or sites	No. events	HPV prevalence, %	Efficacy (95% CI), %
Anal or oral HPV16/18 at the four-year visit	HPV vaccine	192	16	8.3	25.3 (-40.4 to 61.1)
	Control	215	24*	11.2	
Redetection of cervical HPV16/18 (among women with HPV16/18 at enrollment who subsequently cleared their infection)†	HPV vaccine	180	10	5.6	38.9 (-31.7 to 72.9)
	Control	198	18	9.1	
HPV16/18 at two or three sites at the four-year visit	HPV vaccine	192	3	1.6	44.0 (-124.9 to 88.6)
	Control	215	6	2.8	

\* Only two of the 40 total anal/oral events were oral HPV16/18 infections. These two oral HPV16/18 infections were both detected in the control arm, but oral HPV16/18-specific vaccine efficacy did not approach statistical significance given its small sample size. CI = confidence interval; HPV = human papillomavirus.

† Clearance defined as one negative cervical HPV16/18 DNA test after the positive cervical HPV16/18 DNA test.

and oral regions, particularly among those with lower HPV16/18 antibody titers. The lack of statistically significant cervical or multisite VE among those with higher antibody titers may be in

part explained by higher titers partially protecting against cervical HPV16/18 re-infection, which may have reduced the number of cervical HPV16/18 outcomes in the control arm (26,27).

The multisite VE among previously exposed women is important to note given that this study and other randomized trials have been unable to define the location of the original HPV16/18 infection (that induced the HPV16/18 serologic response detected at enrollment). But given the multisite VE suggested in this study, there is now further evidence that HPV vaccination protects some women against acquisition of HPV16/18, including the likely site of the original HPV16/18 infection. However, further examination of the specificity and reliability of the VLP-ELISA is necessary to validate our definition of “previously infected,” as it is possible if false-positive serologic results are common that many in this group may have been truly HPV16/18 naïve (28,29).

In addition to the multisite VE among previously HPV16/18-infected women, this study also suggests some formerly infected women who were HPV16/18 vaccinated may be protected at a subset of their at-risk anatomic sites, as we observed a statistically significantly lower HPV16/18 concordance at two or more anatomic sites in HPV16/18-infected women in the HPV vaccine arm than the control arm. This difference in concordance may be explained by vaccine protection of noninfected sites among women previously exposed to HPV16/18 infection. However, it is less clear whether the vaccine provides women who are cervical HPV16/18 DNA positive at vaccination any protection at other sites given the limited sample size of this group and the lack of oral and anal sampling at enrollment.

The potential vaccine efficacy against noncervical HPV16/18 infection among previously HPV16/18-exposed women is noteworthy given that previous observational studies have suggested the prevalence of anal and oral HPV infections may be similar or even higher at older ages compared with younger individuals in their teens and early 20s (30–32). These findings differ from cervical HPV (33), which is commonly acquired closer to age of sexual debut. While the transmission and natural history of noncervical HPV infection is less understood, the initial studies suggesting a relatively higher incidence of noncervical HPV infection at older ages is noteworthy given our suggestion that HPV vaccination of older individuals may provide some protection against these infections.

While high-income countries recommend routine HPV vaccination in women in their preteen years (age 11–13 years) (34,35), the recommendations for HPV catch-up vaccination and vaccination of older women are more varied. The United States recommends HPV vaccination in women through age 26 years (34), while many other countries do not recommend vaccination for these older age groups (35). While this study and others support lower but statistically significant HPV16/18 vaccine efficacy at older ages, further study (including cost-effectiveness analyses) is necessary to best inform HPV vaccine age range and catch-up policy recommendations. A previous cervical study suggested the risk of cervical precancer is relatively low for women with HPV re-appearance (36), but the clinical relevance of noncervical HPV infections acquired at older ages (acquired sexually or through auto-inoculation) (37–39) needs better understanding.

There were limitations to this study. Specifically, this study's lack of oral and anal sampling at enrollment precluded a completely naïve population, and the outcome measures (one-time detection of cervical, anal, and oral HPV) have lower clinical significance than other commonly used outcomes in HPV vaccine trials (6- to 12-month persistent HPV16/18 infection and high-grade precancers). In addition, some analyses had relatively low power, which limits inference, particularly those related to oral HPV given its low prevalence in this population. However, this study had several strengths, including utilization of the only

randomized trial that examined noncervical HPV in women and an age range of interest (age 18–25 years at vaccination) given the varying recommendations for HPV vaccination in this age group (34,35).

This is the first study to present a combined multisite woman-level HPV16/18 vaccine efficacy among women with and without HPV16/18 exposure at the time of vaccination. These results suggest that multisite HPV16/18 VE is strongest among women never exposed to HPV prior to vaccination, but VE is also evident at one or more anatomic sites among women who likely cleared their HPV16/18 infections prior to HPV vaccination.

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## Notes

The NCI and Costa Rica investigators, not the funders, are responsible for the design and conduct of the study; the collection, management, analysis, and interpretation of the data; and preparation of the manuscript. Registered with Clinicaltrials.gov NCT00128661.

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Investigators in the Costa Rica HPV Vaccine Trial (CVT) Group:

Proyecto Epidemiológico Guanacaste, Fundación INCIENSA, San José, Costa Rica—Bernal Cortés (specimen and repository manager), Paula González (LTFU: co-principal investigator), Rolando Herrero (CVT: co-principal investigator), Silvia E. Jiménez (trial coordinator), Carolina Porras (co-investigator), Ana Cecilia Rodríguez (co-investigator).

United States National Cancer Institute, Bethesda, MD—Allan Hildesheim (co-principal investigator and NCI co-project officer), Aimée R. Kreimer (LTFU: co-principal investigator and NCI co-project officer), Douglas R. Lowy (HPV virologist), Mark Schiffman (CVT: medical monitor and NCI co-project officer), John T. Schiller (HPV virologist), Mark Sherman (CVT: QC pathologist), Sholom Wacholder (statistician).

Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD (HPV Immunology Laboratory)—Ligia A. Pinto, Troy J. Kemp.

Georgetown University, Washington, DC—Mary K. Sidawy (CVT: histopathologist).

DDL Diagnostic Laboratory, the Netherlands (HPV DNA Testing)—Wim Quint, Leen-Jan van Doorn, Linda Struijk.

University of California, San Francisco, CA—Joel M. Palefsky, Teresa M. Darragh.

University of Virginia, Charlottesville, VA—Mark H. Stoler.

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