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Evaluation of Durability of a Single Dose of the Bivalent HPV Vaccine: The CVT Trial

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Abstract

Background: The authors investigated the durability of vaccine efficacy (VE) against human papillomavirus (HPV)16 or 18 infections and antibody response among nonrandomly assigned women who received a single dose of the bivalent HPV vaccine compared with women who received multiple doses and unvaccinated women. **Methods:** HPV infections were compared between HPV16 or 18-vaccinated women aged 18 to 25 years who received one (N = 112), two (N = 62), or three (N = 1365) doses, and age- and geography-matched unvaccinated women (N = 1783) in the long-term follow-up of the Costa Rica HPV Vaccine Trial. Cervical HPV infections were measured at two study visits, approximately 9 and 11 years after initial HPV vaccination, using National Cancer Institute next-generation sequencing TypeSeq1 assay. VE and 95% confidence intervals (CIs) were estimated. HPV16 or 18 antibody levels were measured in all one- and two-dose women, and a subset of three-dose women, using a virus-like particle-based enzyme-linked immunosorbent assay (n = 448). **Results:** Median follow-up for the HPV-vaccinated group was 11.3 years (interquartile range = 10.9–11.7 years) and did not vary by dose group. VE against prevalent HPV16 or 18 infection was 80.2% (95% CI = 70.7% to 87.0%) among three-dose, 83.8% (95% CI = 19.5% to 99.2%) among two-dose, and 82.1% (95% CI = 40.2% to 97.0%) among single-dose women. HPV16 or 18 antibody levels did not qualitatively decline between years four and 11 regardless of the number of doses given, although one-dose titers continue to be statistically significantly lower compared with two- and three-dose titers. **Conclusion:** More than a decade after HPV vaccination, single-dose VE against HPV16 or 18 infection remained high and HPV16 or 18 antibodies remained stable. A single dose of bivalent HPV vaccine may induce sufficiently durable protection that obviates the need for more doses.

Cervical cancer is a leading cause of cancer and cancer death in many countries, particularly in those with a low human development index (1). Human papillomavirus (HPV) vaccines could prevent most cervical cancers, yet uptake is insufficient to make much of an impact on global cancer rates (2). Over the

next 65 years, current vaccination strategies are projected to avert only 3% of the nearly 20 million new cases and 10 million deaths from cervical cancer globally (3).

Compared with the recommended two- and three-dose regimens, single-dose HPV vaccination could reduce costs and

logistical barriers, which consequently could increase vaccine implementation and uptake. We demonstrated in post hoc analyses that a single dose of the bivalent HPV vaccine protected against cervical HPV16 or 18 infection 7 years after initial vaccination compared with age-matched unvaccinated women and elicited a stable systemic antibody response, albeit at lower levels than those induced by three doses, thus necessitating evaluation of virologic endpoints (4).

Modeling efforts suggest durability of the protection is a key component for long-term reduction in HPV prevalence and ultimately cervical cancer (5). Here, we update our nonrandomized analysis of dose-specific HPV vaccine efficacy (VE) against prevalent cervical HPV infection a median of 11 years after vaccination for women who received one, two, and three doses of the bivalent HPV vaccine, compared with unvaccinated women, in the Costa Rica HPV Vaccine Trial (CVT) (6,7).

Methods

Study Participants and Procedures

Participants were enrolled in the publicly funded, community-based, randomized phase III CVT (Clinicaltrials.gov NCT00128661) (7). Between 2004 and 2005, 7466 women aged 18 to 25 years consented and were randomly assigned to receive either the AS04-HPV-16/18 vaccine (Cervarix, GlaxoSmithKline Biologicals, Rixensart, Belgium) or a control hepatitis A vaccine (Havrix, GlaxoSmithKline Biologicals) in a 1:1 ratio at zero, 1, and 6 months and followed up for 4 years. At enrollment and follow-up visits, all participants provided serum, and women who reported intercourse received a pelvic exam, at which time cervical cells were collected for cytology and HPV DNA testing. At the end of the 4-year trial, participants were offered the vaccine they had not received at enrollment and most in the original HPV vaccine arm were invited to participate in a long-term follow-up study (6). During this unblinded follow-up, participants in the original HPV-vaccine arm were followed biennially, and each clinic visit included a pelvic exam with collection of cervical and blood samples. A new unvaccinated control group (UCG, n = 2836) was recruited to replace the control arm, with similar characteristics to trial participants. The 11-year study visit, our final assessment of virologic endpoints given the expected lower rates of HPV acquisition in women in their 30s, finished in August 2017. Protocols were approved by Institutional Review Boards at the US NCI and in Costa Rica; all participants signed informed consent.

Approximately 20% of women received fewer than three doses of their assigned vaccine (8). Reasons for missing vaccine doses were independent of trial arm and mostly due to pregnancy and colposcopic referral (8).

This evaluation focused on HPV-vaccinated women by dose group and the UCG, restricted to women who attended and provided samples at both the 9- and 11-year study visits, to avoid the possibility that discrepancies in the proportion of women who had two visits between the one-, two-, and three-dose groups could bias the conclusions. For virologic endpoints, we analyzed: 112 of 126 women who initially received a single HPV vaccine dose; 62 of 67 women who received two doses at enrollment and 6 months later; 1365 of 1887 women who received three doses; and 1783 of 2189 UCG. For groups 3 and 4, random sampling was used to initially select 50% of each group for testing, and subsequent laboratory testing resulted in additional results being available for inclusion in this analysis (Figure 1).

For serologic endpoints, all available serum from the one- and two-dose women who had serum available at both the 9- and 11-year timepoints (n = 205 and 93) was tested. A random subset of the three-dose group was selected, restricted to women or timepoints included in previous rounds of testing who had sufficient serum (n = 150) (4). The benchmark for natural immunity was inferred from previous testing (4).

Laboratory Methods

HPV DNA
HPV DNA detection and genotyping from cervical specimens were performed at the National Cancer Institute (NCI) Cancer Genomics Research Laboratory using the TypeSeq assay, which detects 51 HPV genotypes based on next-generation sequencing (9,10). A binary result of positive or negative was reported for the human positive control and for each of the 51 HPV types. See the Supplementary Methods (available online) for further details.

HPV Binding Antibody Measurement
HPV16 and HPV18 serum antibody levels were measured by a virus like particle (VLP)-based enzyme-linked immunosorbent assay (ELISA) at the NCI HPV Immunology Laboratory (11–13). The prespecified seropositivity cutoffs for HPV16 and HPV18 were 8 EU (ELISA units)/mL and 7 EU/mL, respectively. Higher cutoffs (HPV16 = 19 EU/mL and HPV18 = 18 EU/mL) (14) were evaluated in a sensitivity analysis. Serology data in the results section of the manuscript are presented using EU/mL; we additionally convert the serology output in years 9 and 11 to International Units in Supplementary Table 1 (available online). Laboratory-blinded replicates (n = 66) were included: the inter-plate coefficient of variation was 2.6% for HPV16 and 3.0% for HPV18. See the Supplementary Methods (available online) for further details.

HPV Neutralizing Antibody Measurement
Secreted alkaline phosphatase-based pseudovirion neutralization assay (SEAP-NA) was used to measure anti-HPV16 and anti-HPV18 neutralizing antibody titers. See the Supplementary Methods (available online) for further details.

Statistical Analysis

The primary analysis compared the percentage of women with a prevalent HPV infection at the 9- and/or 11-year study visit among the analytic groups (one, two, and three HPV vaccine doses and UCG). A prevalent infection was defined as a type-specific HPV infection at either or both the 9- and 11-year study visits and was counted as a single outcome. The secondary analysis compared the percentage of women with an incident HPV infection at the 11-year study visit (ie, no HPV infection at the 9-year study visit) by dose group; this endpoint was defined as an infection (type-specific) being present in year 11 that was not present in year nine (in some cases there may have been intervening clinical management visits; these were ignored); prevalent infections require no prior HPV-infection status information.

For each endpoint, the percentage of women with the endpoint and the VE is reported for each of the dose groups. P values comparing the endpoints in the one- and two-dose groups
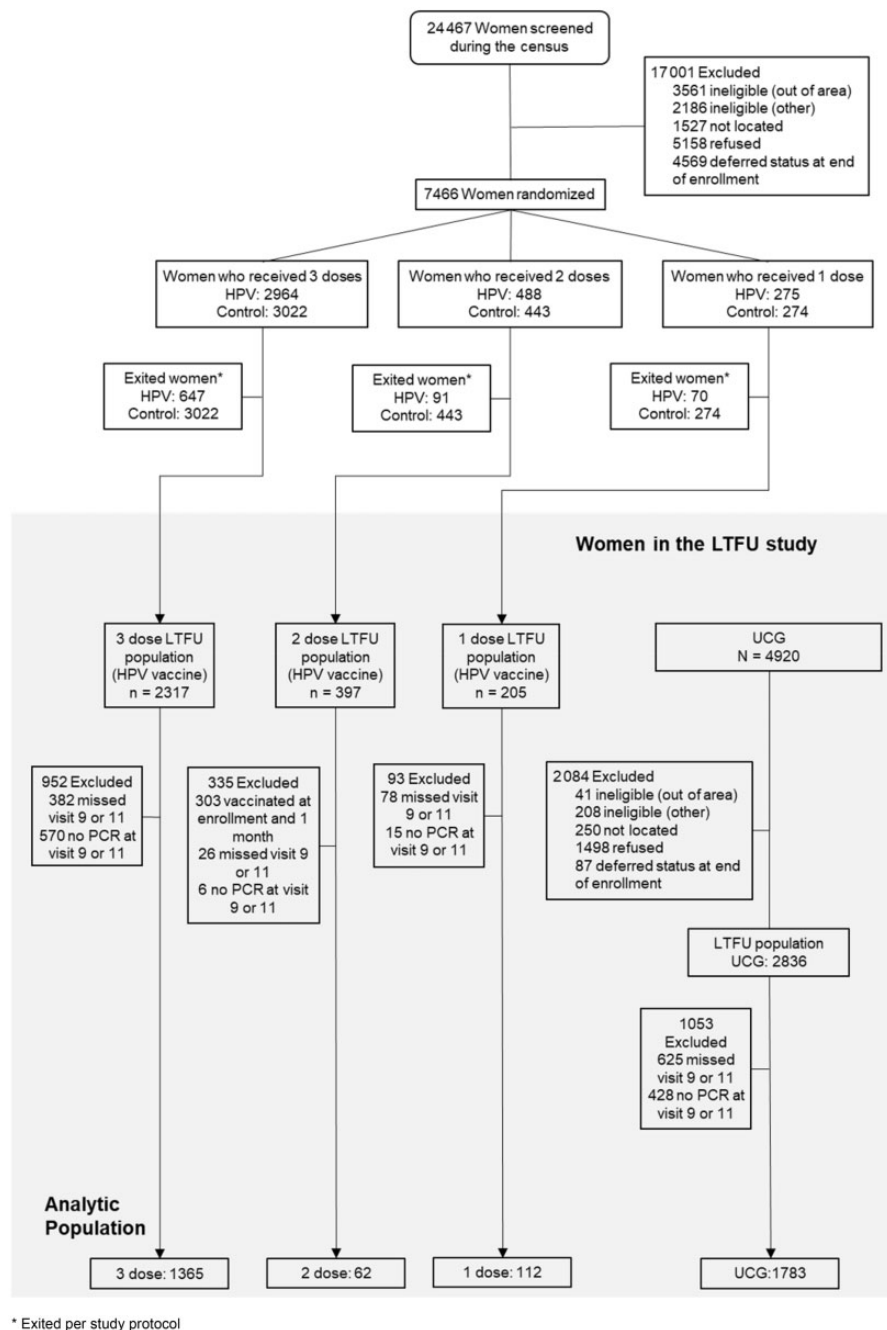
Figure 1. Consolidated standards of reporting trials (CONSORT diagram of women in the nonrandomly assigned extension of the Costa Rica HPV vaccine trial (CVT). This CONSORT diagram shows the women's progress through the randomly assigned phase of the CVT. The CONSORT then details the nonrandomly assigned extension of CVT, the long-term follow-up study (LTFU), where a new unvaccinated control group (UCG) was recruited to replace the control arm, with similar characteristics to trial participants.

with the endpoint in the three-dose group were computed using Fisher's test.

VE was estimated as $1 - P_V/P_U$, where P_v and P_U are, respectively, the prevalence or incidence of infection in the vaccinated and unvaccinated women. Confidence intervals (CIs) for the VE were based on the exact test, conditioning on the total number of events, and using the mid-p adjustment. VE is reported as a composite endpoint for the vaccine types HPV16 or 18 and individually HPV16 and HPV18. Prevalence of carcinogenic HPV types unrelated to HPV vaccination (ie, HPV35, 39, 51, 52, 56, 58, 59) and noncarcinogenic HPV types were evaluated to assess balance in HPV exposure by dose group.

Anti-HPV16 and anti-HPV18 antibody seropositivity are presented for each assay by the three HPV vaccine dose groups using both standard and updated cutoffs. Geometric mean (GM) in ELISA units per milliliter of the serum antibody levels at the 9- and 11-year visits and the ratio of those GMs are reported. We estimate 95% confidence

Table 1. Characteristics at the 11-year study visit among the HPV-vaccinated women, by vaccine dose group, and HPV-unvaccinated women in the CVT

| Characteristic* | Vaccine doses, No. | | | | P‡ |
	3 No. (%)	2 (0 and 6)† No. (%)	1 No. (%)	Unvaccinated No. (%)	
Age, y					
26–32	683 (50.0)	32 (51.6)	50 (44.6)	937 (52.6)	.26
33–38	682 (50.0)	30 (48.4)	62 (55.4)	846 (47.4)	
Sex partners, no.					
0–1	224 (16.4)	12 (19.4)	17 (15.2)	322 (18.1)	.60
2	202 (14.8)	7 (11.3)	19 (17.0)	288 (16.2)	
3+	939 (68.8)	43 (69.4)	76 (67.9)	1173 (65.8)	
Pregnancies, no.					
0	162 (11.9)	7 (11.3)	5 (4.5)	129 (7.2)	<.001
1	363 (26.6)	18 (29.0)	25 (22.3)	351 (19.7)	
2+	840 (61.5)	37 (59.7)	82 (73.2)	1303 (73.1)	
Oral contraceptive use					
Unknown	0	0	0	2	<.001
No	88 (6.4)	2 (3.2)	5 (4.5)	203 (11.4)	
Yes	1277 (93.6)	60 (96.8)	107 (95.5)	1578 (88.6)	
Smoking status					
Unknown	1	0	0	2	
Never	1091 (80.0)	49 (79.0)	98 (87.5)	1446 (81.2)	.24
Former	209 (15.3)	12 (19.4)	12 (10.7)	245 (13.8)	
Current	64 (4.7)	1 (1.6)	2 (1.8)	90 (5.1)	
Follow-up visits during unblinded, long-term follow-up phase of study, no.					
0–3	974 (71.4)	48 (77.4)	82 (73.2)	1044 (58.6)	<.001
4	277 (20.3)	8 (12.9)	21 (18.8)	494 (27.7)	
5+	114 (8.4)	6 (9.7)	9 (8.0)	245 (13.7)	

*To accommodate unvaccinated women enrolled later into long-term follow-up, demographic characteristics were compared at the 11-year study visit, using standard contingency table methods. CVT = Costa Rica HPV Vaccine Trial; HPV = human papillomavirus.
†The two doses were administered at time 0 and 6 months, per the vaccine label.
‡Chi-square two-sided P value.

Results

intervals by assuming the log-level or log-ratio is normally distributed and exponentiating the confidence interval for the mean of the log-transformed measure. All P values are two-sided and considered statistically significant at P less than .05. See the Supplementary Methods (available online) for further details.

Participant Characteristics

Median follow-up for the HPV-vaccinated group was 11.3 years (interquartile range [IQR] = 10.9–11.7 years) and did not vary by dose group (all P ≥ .18). From the 4-year study visit (when the UCG was enrolled), the UCG had slightly less follow-up time (median 6.3 years; IQR = 5.9–6.7 years) compared with the HPV-vaccinated groups (one dose: 6.6 years, two doses: 6.6 years, three doses: 6.6 years). At the final visit, the vaccinated group and the UCG were similar by age, sex partners, and smoking (all P ≥ .24) and differed in pregnancies, OC use, and number of study visits (Table 1).

Virologic Endpoints

VE against prevalent HPV16 or 18 infections more than a decade after initial vaccination was high regardless of dose group (Table 2). VE was 80.2% (95% CI = 70.7% to 87.0%) for three doses, 83.8% (95% CI = 19.5% to 99.2%) for two doses, and 82.1% (95% CI = 40.2% to 97.0%) for a single dose. No statistically significant differences in VE or infection rates were present across dose groups. The background prevalence of nonvaccine carcinogenic HPV genotypes (HPV35, 39, 51, 52, 56, 58, 59) and noncarcinogenic HPV types were similarly high in the vaccinated arms (by dose group) and the UCG, suggesting the groups were at similar risk for HPV infection (Table 2).

No statistically significant differences were observed in point estimates for VE against incident HPV16 or 18 infection at the study visit that occurred, on average, 11 years after initial vaccination; 95% confidence intervals were wide, especially for the one- and two-dose groups, because few incident infections were observed. VEs were qualitatively different between dose groups: 84.9% (95% CI = 69.8% to 93.2%) for the three-dose group, 58.4% (95% CI = −110.9% to 97.9%) for the two-dose group, and 53.9% (95% CI = −57.1% to 92.4%) for the one-dose group. Further, likely due to small sample sizes for the one- and two-dose groups, the estimated VEs were not statistically different from 0 (Table 2).

Immunologic Endpoints

Using our standard threshold for positivity based on the ELISA assay, 100% of HPV-vaccinated women remained seropositive at years nine and 11 regardless of number of doses received. In the sensitivity analysis using higher cutoffs, seropositivity at the 9-year study visit was 97.7% (95% CI = 93.8% to 99.4%) for HPV16 and 96.1% (95% CI = 91.6% to 98.6%) for HPV18 among

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Table 2. HPV VE using prevalent and incident HPV infections as the endpoint among women tested at both years nine and 11

HPV endpoint	Unvaccinated (n = 1783)		3 Doses (n = 1365)			2 Doses (0 and 6)* (n = 62)				1 Dose (n = 112)			
	No. HPV +	% HPV+ (95% CI)	No. HPV +	% HPV+ (95% CI)	VE, % (95% CI)	No. HPV +	% HPV+ (95% CI)	VE, % (95% CI)	P†	No. HPV +	% HPV+ (95% CI)	VE, % (95% CI)	P†
Prevalent HPV infections at year 9 or 11													
16 or 18	178	10.0 (8.7 to 11.4)	27	2.0 (1.3 to 2.8)	80.2 (70.7 to 87.0)	1	1.6 (0.1 to 7.7)	83.8 (19.5 to 99.2)	1.00	2	1.8 (0.3 to 5.8)	82.1 (40.2 to 97.0)	1.00
16	111	6.2 (5.2 to 7.4)	22	1.6 (1.0 to 2.4)	74.1 (59.7 to 84.0)	1	1.6 (0.1 to 7.7)	74.1 (−30.0 to 98.7)	1.00	2	1.8 (0.3 to 5.8)	71.3 (3.3 to 95.2)	.70
18	74	4.2 (3.3 to 5.2)	6	0.4 (0.2 to 0.9)	89.4 (77.0 to 95.8)	0	0.0§ (0.0 to 4.7)	100.0 (−18.8 to 100)	1.00	0	0.0§ (0.0 to 2.6)	100.0 (34.2 to 100)	1.00
Carcinogenic HPV unrelated to vaccine‡	426	23.9 (22.0 to 25.9)	339	24.8 (22.6 to 27.2)	−3.9 (−19.9 to 9.9)	19	30.6 (20.2 to 42.9)	−28.3 (−98.8 to 21.2)	.30	25	22.3 (15.3 to 30.7)	6.6 (−37.6 to 38.8)	.65
Noncarcinogenic	898	50.4 (48.0 to 52.7)	722	52.9 (50.2 to 55.5)	−5.0 (−15.8 to 4.8)	37	59.7 (47.2 to 71.3)	−18.5 (−62.8 to 15.9)	.30	68	60.7 (51.4 to 69.4)	−20.5 (−53.3 to 6.4)	.12
Incident HPV infections at year 11													
16 or 18	69	3.9 (3.1 to 4.9)	8	0.6 (0.3 to 1.1)	84.9 (69.8 to 93.2)	1	1.6 (0.1 to 7.7)	58.4 (−110.9 to 97.9)	.33	2	1.8 (0.3 to 5.8)	53.9 (−57.1 to 92.4)	.17
16	37	2.2 (1.6 to 2.9)	8	0.6 (0.3 to 1.1)	72.6 (43.2 to 88.1)	1	1.6 (0.1 to 7.7)	25.5 (−286.6 to 96.4)	.33	2	1.8 (0.3 to 5.8)	17.5 (−189.0 to 86.6)	.17
18	32	1.8 (1.3 to 2.6)	0	0.0§ (0.0 to 0.2)	100.0 (87.4 to 100)	0	0.0§ (0.0 to 4.7)	100.0 (−175.6 to 100)	N/A	0	0.0§ (0.0 to 2.6)	100.0 (−52.6 to 100)	N/A
Carcinogenic HPV unrelated to vaccine‡	198	11.1 (9.7 to 12.6)	142	10.4 (8.9 to 12.1)	6.3 (−16.1 to 24.6)	6	9.7 (4.0 to 19.0)	12.9 (−84.1 to 65.1)	1.00	16	14.3 (8.7 to 21.7)	−28.6 (−109.1 to 25.2)	.20
Noncarcinogenic	514	28.8 (26.8 to 31.0)	425	31.1 (28.7 to 33.6)	−8.0 (−22.8 to 5.0)	14	22.6 (13.5 to 34.2)	21.7 (−29.4 to 55.7)	.16	44	39.3 (30.6 to 48.6)	−36.3 (−83.7 to 0.9)	.09

*The two doses were administered at time 0 and 6 months, per the vaccine label. CI = confidence interval; HPV = human papillomavirus; N/A = not applicable; VE = vaccine efficacy.
†P value comparing rate in specified dose group to rate in three-dose group.
‡Includes HPV35, 39, 51, 52, 56, 58, and 59.
§Cell with zero observations.

Table 3. Distributions of ELISA serum antibody concentration levels and SEAP neutralization titers for HPV16 and HPV18 at years nine and 11

Assay	Metric	Three doses GM (95% CI)	IQR	Two doses (0/6 mo) GM (95% CI)	IQR	One dose GM (95% CI)	IQR
HPV 16 ELISA antibody concentration							
ELISA antibody	Year 9	699 (606 to 807)	394–1265	414 (328 to 524)	197–813	172 (141 to 209)	83–319
concentration, EU/mL	Year 11	664 (570 to 772)	383–1309	340 (267 to 434)	170–684	176 (145 to 214)	84–370
	Change, % (95%CI)	−1.6 (–8.6 to 5.5)		−17.9 (–28.5 to –7.4)		0.3 (–13.4 to 13.9)	
	Ratio of GM	0.95 (0.89–1.01)		0.82 (0.76–0.89)		1.03 (0.94–1.13)	
HPV18 ELISA antibody concentration							
ELISA antibody	Year 9	292 (249 to 342)	163–532	210 (171 to 259)	119–426	102 (83 to 125)	48–223
concentration, EU/mL	Year 11	275 (234 to 323)	160–518	194 (156 to 241)	117–384	109 (89 to 133)	51–220
	Change, % (95%CI)	−4.0 (–13.6 to 5.6)		−2.1 (–22.5 to 18.4)		−1.3 (–14.0 to 11.5)	
	Ratio of GM	0.94 (0.88–1.01)		0.92 (0.83–1.02)		1.07 (0.99–1.16)	
HPV 16 SEAP-NA neutralization titer							
SEAP-NA antibody titer	Year 9	1776 (1389 to 2270)	1014–3114	771 (612 to 970)	382–1484	285 (229 to 355)	120–654
	Year 11	1565 (1227 to 1997)	924–2916	626 (491 to 798)	323–1405	285 (233 to 349)	133–608
	Change, % (95%CI)	−10.5 (–21.7 to 0.7)		−17.2 (–28.1 to –6.4)		−11.5 (–29.7 to 6.7)	
	Ratio of GM	0.88 (0.81–0.96)		0.81 (0.75–0.88)		1.00 (0.90–1.11)	
HPV18 SEAP-NA neutralization titer							
SEAP-NA antibody titer	Year 9	856 (645 to 1136)	425–1615	531 (415 to 678)	295–1092	216 (170 to 273)	93–504
	Year 11	792 (588 to 1065)	371–1573	489 (371 to 644)	217–1080	233 (184 to 296)	96–551
	Change, % (95%CI)	−2.6 (–15.9 to 10.6)		14.6 (–33.2 to 62.4)		6.8 (–7.4 to 21.0)	
	Ratio of GM	0.92 (0.81–1.06)		0.92 (0.80–1.06)		1.08 (0.98–1.19)	

*CI = confidence interval; ELISA = enzyme-linked immunosorbent assay; GM = geometric mean; IQR = interquartile range; SEAP-NA = secreted alkaline phosphatase-based pseudovirion neutralization assay.

one-dose women, 100% (95% CI = 96.2% to 100%) for HPV16 and 18 for two-dose women, and 100% (95% CI = 97.8% to 100%) for HPV16 and 18 for three-dose women. Seropositivity at the 11-year visit was 96.7% (95% CI = 93.3% to 98.7%) for HPV16 and 92.9% (95% CI = 88.5% to 96.0%) for HPV18 for one-dose women, 98.7% (95% CI = 93.8% to 99.9%) for HPV16 and 100% (95% CI = 96.2% to 100%) for HPV18 for two-dose women, and 100% (95% CI = 97.9% to 100%) for HPV16 and 18 for three-dose women (data not shown). Using the higher cutoff, 0.0% and 4.2% of one-dose women went from HPV16 and HPV18 seropositive to seronegative between years nine and 11, respectively.

Based on ELISA, antibody concentration levels for HPV16 and HPV18 remained relatively constant between years nine and 11 for all dose groups, although GMs did show a slight decrease for three- and two-dose women along with a slight but nonstatistically significant increase for one-dose women (Table 3). Among women in the three-, two-, and one-dose groups, the changes in the GMs of the HPV16 antibody concentration levels were −1.6% (95% CI = −8.6%% to 5.5%), −17.9% (95% CI = −28.5% to −7.4%), and +0.3% (95% CI = −13.4% to 13.9%), respectively. In the same groups, the change in the GMs of the HPV18 antibody concentration levels, respectively, were −4.0% (95% CI = −13.6% to 5.6%), −2.1% (95% CI = −22.5% to 18.4%), and −1.3% (95% CI = −14.0% to 11.5%). Using SEAP-NA, results were similar: percent change from year nine to year 11 was only statistically significant for HPV16 in the two-dose group and ratios of the GMTs that excluded 1.0 were HPV16 by ELISA for the two-dose group and HPV16 by SEAP-NA for the two- and three-dose groups.

We evaluate trends in GMs across the six study visits (ie, years two, three, four, seven, nine, and 11) in Figure 2 and Supplementary Figures 1–3 (available online). As expected, there were small decreases in antibody levels between years four and 11 in the two- and three-dose women. In one-dose women, there was no change (P = .72) in HPV16 antibody levels over time and actually a small, statistically significant increase in the HPV18 antibody levels over time, with an average per-year increase in the log-level of 0.017 (95% CI = 0.005 to 0.029) driven by

the modest shift between years one to four and years seven to 11 (Figure 2). Adjustment for testing batch did not qualitatively modify results. This effect disappears when limiting analyses to women with measurements at all years. In this group, the average per-year increase in the log-level of the HPV18 titer was 0.015 (95% CI = −0.002 to 0.032) (Supplementary Figure 3, available online). HPV seropositivity based on the higher threshold and comparing years nine and 11, greater than 90% of HPV-vaccinated women were HPV16 and 18 seropositive at both timepoints, and 2.5% of one-dose HPV-vaccinated women were HPV16 and 18 seronegative at both timepoints (Supplementary Table 2, available online).

Over the course of the study, the NCI Immunology Laboratory conducted three major batches of serologic testing by ELISA [4-year (15), 7-year (4), and 11-year analyses]. HPV16 and 18 antibody results were compared across batches; the reproducibility was excellent (Supplementary Table 3 and Supplementary Figure 4, available online). Correlations between ELISA and SEAP-NA from the final batch of testing, collapsing across years nine and 11, were also very high (Supplementary Table 4, available online).

Extensive analyses were conducted to confirm antibody stability over time by dose (Supplementary Figures 5–9, available online). Supplementary Figure 6 (available online) shows the HPV antibody levels in most women do not qualitatively change over time and only a minority of one-dose women experienced a qualitative increase, suggesting that sexual exposure to HPV16 or 18 does not boost antibodies in the majority of women and thus does not explain the observed persistence in antibody levels.

Discussion

We present our final assessment of HPV infection, more than a decade after the initial bivalent HPV vaccination, in this post hoc evaluation of HPV vaccine protection for women who
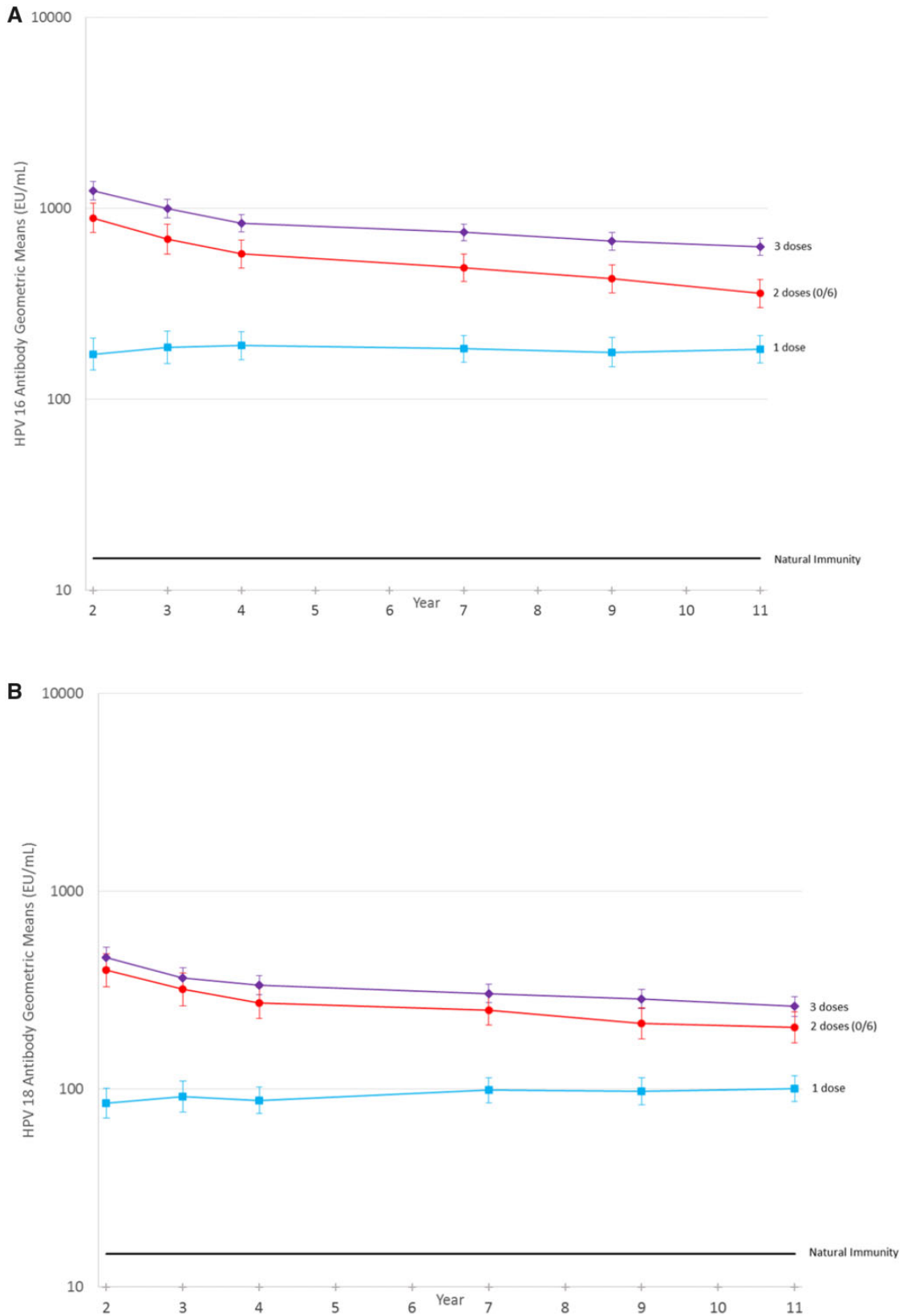
Figure 2. Human papillomavirus (HPV)16 (graph A) and HPV18 (graph B) antibody levels over time by number of doses received. A linear mixed model using all titer results including replicate testing was used to calculate the Geometric mean titers (GMTs) and 95% confidence intervals (CIs) for single-dose women using continuous time, adjusted by testing batch and using random effects to account for the correlation within a patient and within replicate testing of a visit. For HPV16, the average per-year change in the log titer level for the 221 women with one dose is −0.002 (95% CI = −0.015 to 0.011, P = .72). For HPV18, the average per-year change in the log titer level for the 221 women with one dose is 0.017 (95% CI = 0.005 to 0.029, P = .005).

received one, two, and three doses compared with UCG. High VE against HPV16 or 18 infections was similar for all dose groups, suggesting that the protection afforded by even a single dose may be sufficient to produce a lifelong impact on cancer prevention. Although these women were not randomly assigned to dose group and differences were observed for OC use and pregnancy, the similarity in HPV prevalence for nonvaccine carcinogenic and noncarcinogenic types indicates their risk for HPV exposure was similar, allaying concerns that bias or differential HPV exposure could explain these findings.

VE was assessed among all women with available test results in years nine and 11 using an endpoint of prevalent HPV infection to maximize power (instead of incident persistent HPV infection). VE analyses in such cohorts and using such endpoints result in attenuated VE estimates. Nonetheless, VE among women who received a single HPV vaccine dose was 82% and was not statistically different from the observed VE for women who received three doses, the standard of care in this age group. It is important to note that the lower bound of the 95% confidence interval of the VE estimate for the one-dose group was 40.2%, meaning that a substantially lower VE cannot be ruled out; tighter confidence intervals were not possible given the small sample size of the single-dose group. Notably, all HPV-vaccinated women, regardless of number of doses received, remained HPV16 and 18 seropositive using our traditional cutoff more than a decade after initial vaccination, and the average drop in antibody levels between years nine and 11 was modest. Even with the more stringent cutoff, very high seropositivity was observed. Surpassing the 10-year benchmark is important given modeling efforts showing that a single-dose routine vaccination program requires durable protection to avert a substantial number of cervical cancers (16).

Other antivirion antibody responses induced by viral infection or live viral vaccines, which present the same type of high-density repetitive display of surface epitopes as HPV VLPs, have been shown to persist at essentially constant levels for many decades (17). Yet the sustained immunological responses observed among single-dose HPV-vaccinated participants were unexpected because it has not been observed with other subunit vaccines. For example, anti-Hepatitis B (HB) positivity in young adults (median age of 25 years) after single-dose HB vaccination was 4% and dropped to 0% after 2 years (18), with antibody levels below the threshold considered necessary for protection against HBV infection (19). However, the subviral particles that comprise the HBV vaccine may not be sufficiently "virus-like" to efficiently induce durable antibody responses. Antibody responses to a single dose of the HPV vaccine more closely resemble those to a live virus infection, which can persist indefinitely at a relatively steady-state level (20). In keeping with the idea first proposed by Bachman and Zinkernagel, we speculate that the rigid and densely ordered display of antigens on the VLP surface, as with the display of antigens on many authentic virions, is specifically recognized as foreign by the humoral immune system, with the cross-linking of the B-cell receptors on cognate naïve B cells by the repetitive antigen inducing exceptionally strong activation and survival signals and leading to the generation of long-lived plasma cells that continuously secrete antibodies in the absence of further antigen stimulation (17,21,22). Of note, while we document protection with a single vaccine dose against homologous HPV types in this work, complementary findings against heterologous types show strong efficacy with one, two, and three doses (23).

The main limitations of our study are the small sample sizes of the one- and two-dose groups, which are fixed and thus have limited power to detect small differences in HPV attack rates by dose. Because women were not randomly assigned to dose group, we have done extensive work to rule out bias (22,24), including documenting similar antibody responses by dose group 1 month after the initial dose, and balance in the attack rate of non-16 or 18 HPV types by dose in years four, seven, nine, and 11. Yet these data do not afford the same reassurance against selection bias and level of evidence as a randomized trial comparing single with multiple doses. To overcome this, we implemented the Scientific Evaluation of One or Two Doses of the Bivalent or Nonavalent Prophylactic HPV Vaccines (the ESCUDDO study), a randomized clinical trial to definitively determine the protection afforded by single-dose regimens of the HPV vaccines (ClinicalTrials.gov identifier: NCT03180034). ESCUDDO is a four-arm randomized, noninferiority efficacy trial in Costa Rica that aims to evaluate whether, in adolescent girls, one dose or two doses of the bivalent or nonavalent vaccines can confer strong, durable protection against persistent HPV infections (sample size approximately 20 000). Analyses will be conducted to estimate VE vs no vaccination using a concurrent population survey of comparable, initially unvaccinated females in the same region; results are anticipated in 2025.

From the global perspective, women who are at the greatest lifetime risk of cervical cancer are not being vaccinated (3). Our nonrandomized data suggest that a single dose of the HPV vaccine protects against HPV infection a decade after vaccination, with documented stabilization of antibody responses. Given the small number of women who received a single dose, we cannot be certain that one dose will be as protective against HPV infection as two or three doses. But the very strong differences between the one-dose and the unvaccinated groups are unlikely to be explained by biases or chance findings, providing compelling evidence that a single dose of the HPV vaccine is superior to not vaccinating.

Funding

Notes

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. JTS and DRL report that they are named inventors on US Government-owned HPV vaccine patents that are licensed to GSK and Merck and for which the NCI receives licensing fees. They are entitled to limited royalties as specified by federal law. The other authors declare that they have no conflicts of interest. Where authors are identified as personnel of the International Agency for Research on Cancer, World Health Organization, the authors alone are responsible for the views expressed in this article, and they do not necessarily represent

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