Prevention of Persistent Human Papillomavirus Infection by an HPV16/18 Vaccine: A Community-Based Randomized Clinical Trial in Guanacaste, Costa Rica

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ABSTRACT

Target groups for human papillomavirus (HPV) vaccination are controversial. We evaluated vaccine efficacy (VE) against 1-year persistent infection, stratified by age and sexual behavior, among young women in Costa Rica**.** We randomized 7,466 healthy women 18 to 25 years of age to HPV16/18 or hepatitis A vaccine (follow-up, 50.4 months). According-to-protocol (ATP) cohorts included compliant HPV-negative women; intention-to-treat (ITT) included all randomized women. ATP VE was 90.9% (95% CI, 82.0–95.9) against HPV16/18 infections, 44.5% against HPV31/33/45 (95% CI, 17.5–63.1), and 12.4% (95% CI, −3.2 to 25.6) against any oncogenic infection. Overall ITT VE against HPV16/18 infections was 49.0%, but ATP and ITT VE almost reached 100% in year 4 of follow-up. ATP efficacy against HPV16/18 was similar by age, but ITT VE was greatest among youngest women (68.9% among those 18–19 years of age; 21.8% among those 24–25 years of age) and 79.8% among virgins. Among previously unexposed women, vaccination is highly efficacious against HPV16/18 and partially against HPV31/33/45. Vaccination is most effective in women and girls before they initiate sexual activity, with programmatic and individual decision implications.

SIGNIFICANCE: In an independent trial of the bivalent AS04-adjuvanted HPV16/18 vaccine (Cervarix) conducted among young women in Costa Rica, we confirmed the high efficacy against HPV16/18 persistent infection and partial cross-protection against HPV31/33/45. Furthermore, efficacy data suggest that the benefit of HPV vaccination is maximal when the vaccine is given to young women before they initiate sexual activity. *Cancer Discovery; 1(5):* 408–19. *©2011 AACR.*

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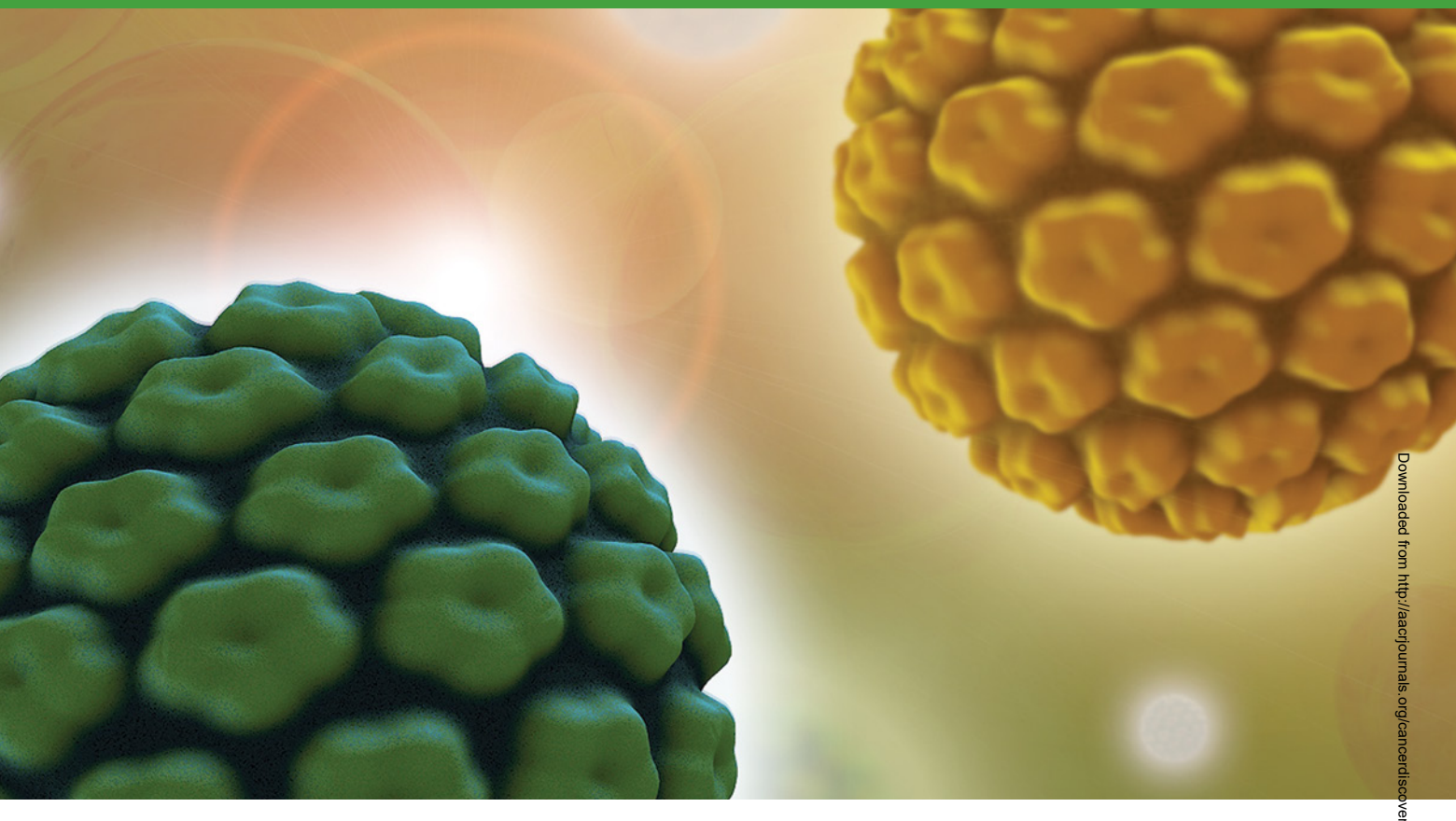

INTRODUCTION

Human papillomavirus (HPV) vaccines have enormous potential in the control of cervical cancer, and developed countries are vaccinating adolescent girls to prevent cervical neoplasia. However, the worldwide cervical cancer burden (500,000 cases annually) will only decrease with high vaccination coverage in developing countries, where most cases (85%) occur (1). In this context, target ages and population groups to maximize reduction in morbidity, treatment, and mortality are still controversial.

The two vaccines based on L1 virus-like particles licensed worldwide, quadrivalent human papillomavirus (types 6, 11, 16, 18) vaccine (Gardasil; Merck and Co., Inc.) (2, 3) and bivalent human papillomavirus (types 16 and 18) vaccine (Cervarix; GlaxoSmithKline Biologicals) (4, 5), are highly protective against cervical neoplasia caused by HPV types 16 and 18 (HPV16 and 18) among women without current or past infection with these types. There is also evidence of limited cross-protection against HPV31, 33, 45, and possibly others (5–7). However, vaccination does not increase clearance or decrease progression of established infections (8, 9).

The ultimate goal is the prevention of cervical cancer, but trials with that end point are impractical. The choice of trial end points has been intensively debated (10). Regulatory authorities required histopathological outcomes, namely cervical intraepithelial neoplasia grade 2 or greater (CIN2+) (in effect, mainly CIN2), as cancer surrogates in licensure trials.

Although HPV infection is a necessary cause of cervical cancer, acute infection is extremely common and usually clears within months (11). Persistent oncogenic HPV infection, which is less frequent, is a much better end point than incident infection and, in some respects, a better surrogate marker than CIN2 because infection can be measured with high reproducibility (12), whereas CIN2 is subject to significant histologic misclassification (13). Also, attribution of the HPV type that caused a CIN2+ is difficult when multiple types of infections are present, as is common (14).

The evaluation of vaccine efficacy (VE) and potential impact in population subgroups can assure maximum benefit from high-cost programs in different settings. Developing countries with limited resources are considering whether investment in this preventive measure is worthwhile. In developed countries, a benefit is uncertain for older women born in earlier cohorts and those who miss vaccination as adolescents, particularly in the United States, where uptake of the vaccine in adolescents is limited (15).

We report here on efficacy of an HPV16/18 ASO4-adjuvanted vaccine (Cervarix) in a large community-based clinical trial [registered at clinicaltrials.gov (NCT00128661)] in a high-incidence area of Costa Rica (16), with 1-year persistence of a cervical HPV infection as an end point, including estimates of VE by age, sexual behavior, and previous exposure to individual HPV types. We present results for both intention-to-treat (ITT) cohorts, reflecting real-world efficacy, and according-to-protocol (ATP) cohorts, as a proxy for an ideal in which women are fully vaccinated before exposure so they can receive maximum benefit.

RESULTS

Figure 1 presents the trial profile diagram. Of nearly 25,000 women screened, 7,466 women were randomized,

with 3,727 in the vaccine arm and 3,739 in the control arm (ITT cohort for HPV16/18). The ATP cohort comprised 2,635 and 2,677 women in the vaccine and control arms, respectively. The 7,466 women represented 59.1% of 12,624 potentially eligible women (considering those with recruitment deferred beyond the enrollment period for different reasons as noneligible) and 30.5% of all 24,467 women screened from the census.

Age, study clinic, presence and number of individual HPV types detected, and baseline cytology were similar in the two arms (Supplementary Table S1). As noted previously (8), HPV16 was more common at baseline in the vaccine than in the control arm (6.0% vs. 7.1%, $P = 0.05$). For this analysis, women in the ATP cohort for HPV16/18 had accumulated 10,268 and 10,472 person years in the vaccine and control arm, respectively, with a median follow-up time of 50.4 months. Total follow-up time, number of visits, maximum time between tests, and number of annual, semiannual, or colposcopy visits were similar by arm (data not shown). More than 90% of eligible women attended their corresponding visits and provided specimens.

Estimated VE against HPV16/18 was 90.9% (95% CI, 82.0–95.9) in the ATP cohort and 49.0% (95% CI, 38.1–58.1) in the ITT cohort (Table 1). The efficacy against HPV31/33/45, for which previous evidence of protection exists, was 44.5% (95% CI, 17.5–63.1) in the ATP cohort. Efficacies against other oncogenic types combined were not significant. The overall efficacy against all oncogenic types was approximately 10% in both the ATP and ITT analyses. Considering individual A9-species HPV types in ATP cohorts, protection against the HPV16 target was 86.5% (95% CI, 72.9–94.0), with significant cross-protection against HPV31 (45.7%; 95% CI, 8.2–68.6). There was a nonsignificant cross-protection (37.3%; 95% CI, −51.4 to 75.3) against HPV33 but not against other types in this species (Supplementary Table S2). In species A7, efficacy against HPV18 persistent infection was 100% (95% CI, 90.7–100.0), with nonsignificant cross-protection for closely related HPV45 (52.0%; 95% CI, −9.8 to 80.4). The other types in this species had nonsignificant negative estimates of efficacy. In species A5 and A6, the only noteworthy finding was an increase in persistent infection with HPV51 (A5) in the HPV arm (VE: −63.9%; 95% CI, −150.7 to −8.2).

Table 2 presents ATP efficacy against HPV16 by baseline HPV16 serology status. Rates of "breakthrough" persistent infections in the HPV arm were greater among seropositive patients than seronegative patients, although in the control arm, the rate of infections was lower in the seropositive patients. Thus, efficacy was greater than 90% among HPV16 seronegative women but only 50% among the seropositive women. Interestingly, efficacy against HPV16 was similar among HPV18 seronegative and seropositive women.

Efficacy in the ATP cohort was similar regardless of vaccination age (P for trend 0.362; Table 3); however, in the ITT cohort, VE decreased from 68.9% (95% CI, 53.1–79.9) for women 18–19 years of age to 21.8% (95% CI, −16.9 to 47.9) among 24- to 25-year-old women (P for trend = 0.005). Corresponding rate reductions per 100 women vaccinated decreased from 5.2 (95% CI, 3.6–6.6) to 1.6 (95% CI, −1.0 to 4.0). Similarly, in the ITT cohort, efficacy was greatest among virgins at enrollment (79.8%; 95% CI, 44.9–94.1), with decreasing efficacy with increasing time since first sexual intercourse (Table 4) and increasing number of sexual partners (Table 5). When we considered stratification of the ITT results by time since first sexual intercourse and number of sexual partners, we found that virgins, despite high VE, had

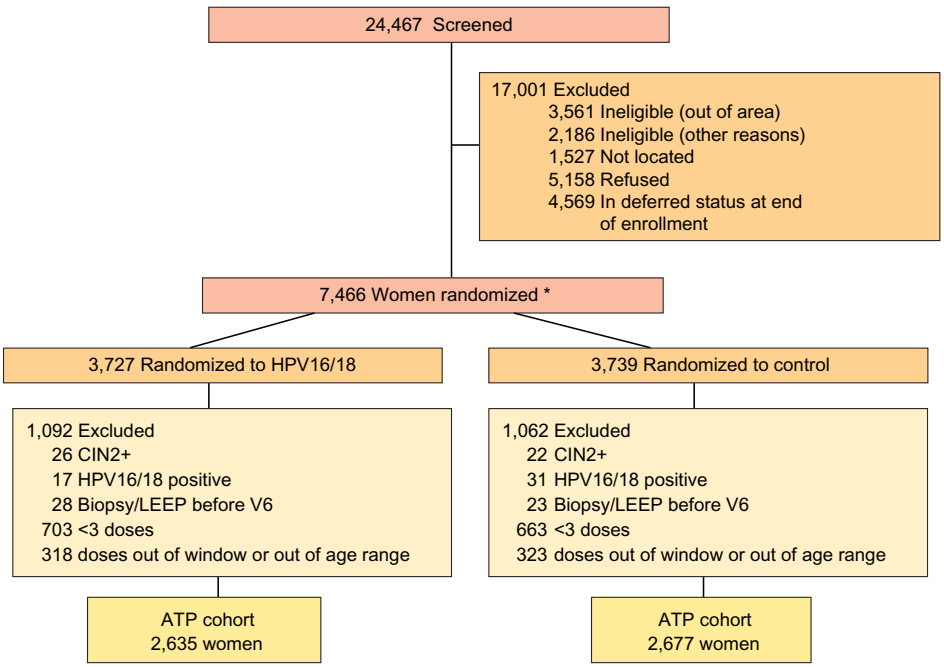

Figure 1. Trial profile. *Four women received discordant vaccines (one woman was enrolled twice and received 3 doses of each vaccine and three women received 2 doses of one vaccine and one dose of the other vaccine). For the aim of this analysis, the women were assigned to the group for which the first dose was given. LEEP, loop electrosurgical excision procedure.

Table 1. VE against 1-year persistence of different combinations of HPV types

		ATP analysis[a]					ITT analysis[b]				
HPV type	Arm	Women in ATP cohort, *N*	Women with events, *n*	Rate per 100 women, *n* (95% CI)	Rate reduction/ 100 women, *n* (95% CI)	Efficacy, % (95% CI)	Women in ITT cohort, *N*	Women with events, *n*	Rate per 100 women, *n* (95%CI)	Rate reduction/ 100 women, *n* (95%CI)	VE, % (95% CI)
HPV16,18	Vaccine	2,635	8	0.3 (0.1–0.6)	3.0 (2.5–3.3)	90.9[c] (82.0–95.9)	3,727	153	4.1 (3.5–4.8)	3.9 (2. 9–5.0)	49.0[d] (38.1–58.1)
	Control	2,677	89	3.3 (2.7–4.1)			3,739	301	8.1 (7.2–9.0)		
HPV31, 33, 45	Vaccine	2,642	37	1.4 (1.0–1.9)	1.1 (0.4–1.8)	44.5 (17.5–63.1)	3,727	150	4.0 (3.4–4.7)	0.7 (–0.2 to 1.7)	15.5 (–5.0 to 32.0)
	Control	2,695	68	2.5 (2.0–3.2)			3,739	178	4.8 (4.1–5.5)		
Other oncogenic types	Vaccine	2,643	230	8.7 (7.7–9.8)	–1.0 (–2.6 to 0.5)	–13.4 (–36.9 to 6.0)	3,727	559	15.0 (13.9–16.2)	–0.2 (–2.0 to 1.5)	–1.4 (–14.1 to 9.8)
	Control	2,697	207	7.7 (6.7–8.7)			3,739	553	14.8 (13.7–16.0)		
Any oncogenic type	Vaccine	2,643	267	10.1 (9.0–11.3)	1.4 (–0.3 to 3.2)	12.4 (–3.2 to 25.6)	3,727	764	20.5 (19.2–21.8)	2.6 (0.5–4.7)	11.3 (2.2–19.5)
	Control	2,697	311	11.5 (10.4–12.8)			3,739	864	23.1 (21.8–24.5)		

[a]The ATP cohort includes women who received all 3 doses within protocol-defined windows, complied with the protocol during the vaccination period, did not have a biopsy or treatment (loop electrosurgical excision procedure [LEEP]) before the 6-month visit, and were HPV DNA negative (by PCR) for at least one of the HPV types in the end point at enrollment and at the 6-month visit.

[b]ITT cohorts include all women randomized and vaccinated, regardless of prevalence of infection and follow-up visits.

[c]One-sided *P*-value for test of VE equals 0 against the alternative that VE is greater than 0 is less than 10^{-17}.

[d]One-sided *P*-value for test of VE equals 0 against the alternative that VE is greater than 0 is less than 10^{-11}.

Table 2. VE (ATP) against 1-year persistence with HPV16 stratified by HPV16 and HPV18 serology at enrollment

HPV serology[a,b]	Arm	Women, *N*	Women with events, *n*	Rate per 100 women	95% CI	Rate reduction per 100 women	95% CI	VE, %	95% CI
HPV16 serology negative	Vaccine	1,875	4	0.2	0.1–0.5				
	Control	1,856	51	2.7	2.1–3.6	2.5	2.0–2.8	92.2	80.3–97.6
HPV16 serology positive	Vaccine	558	4	0.7	0.2–1.7				
	Control	551	8	1.5	0.7–2.7	0.7	–0.5 to 1.7	50.6	–63.3 to 87.0
HPV18 serology negative	Vaccine	1,853	4	0.2	0.1–0.5				
	Control	1,854	44	2.4	1.8–3.1	2.2	1.6–2.4	90.9	76.7–97.2
HPV18 serology positive	Vaccine	563	3	0.5	0.1–1.4				
	Control	541	14	2.6	1.5–4.2	2.1	0.6–2.9	79.4	33.5–95.3

[a]The stratification by HPV16 serology excludes 31 and 45 subjects without HPV16 serology results from the vaccine and control arm, respectively.

[b]The stratification by HPV18 serology excludes 48 and 57 subjects without HPV18 serology from the vaccine and control arm, respectively.

a lower rate reduction than sexually active women because they can only contribute outcomes after the initiation of sexual activity and therefore have less observation time. Among sexually active women, rate reductions, such as VE, decreased with time since first sexual intercourse. However, rate reductions increased with the number of sexual partners despite decreasing VE, as a consequence of the greater attack rate with increasing number of partners.

We also investigated VE according to time between vaccination and incidence of persistent infections (Table 6). In the ATP analysis, VE against HPV16/18 increased with time since enrollment to 100% after 34 months. In the ITT analysis, efficacy also increased with follow-up from only 16% in the first 2 years to more than 90% after 46 months. A similar effect was observed when we considered efficacy against HPV31, 33, and 45 combined: ATP efficacy changed from 41.7% (95% CI, −31.3 to 75.4) 10–22 months after vaccination to 57.6% (95%CI, −31.9 to 88.5) after 46 months. In the ITT analysis, corresponding VE went from −19.4% (95% CI, −64.9 to 13.3) to 53.1% (95% CI, 8.0 to 77.1).

In an effort to compare the VE to prevent 12-month persistent infections with VE to prevent 6-month persistent infections, we also calculated VE against that outcome, including the same stratified analyses (Supplementary Tables S3–S9). The results were very similar, although there is more statistical power. In this context, it is noteworthy that HPV31 was no longer the only nonvaccine HPV type with significant protection. The VE to prevent 6-month infection with HPV45 was 73.0% (95% CI, 45.3–87.8). Among women who were HPV DNA positive at enrollment, we did not detect significant efficacy against persistent infection with any of the HPV types investigated (Supplementary Table S10).

We also analyzed the 600 subjects excluded from ATP because they received at least 1 of the 3 vaccine doses outside the ATP windows. Estimates of VE against HPV16/18 with the use of similar exclusion criteria as those for the ATP analysis and among all women (ITT) produced results similar to the respective analyses among women who received their 3 doses within the windows (Supplementary Table S11).

DISCUSSION

Results from this independent trial support the strong protective effect of Cervarix against 12-month HPV16/18 persistent infections in the ATP cohort (5). Protection was close to 90% against these two types, which are responsible for approximately 70% of cervical cancers (17). In addition, we observed nearly 50% cross-protection against HPV31/33/45, which are associated with approximately 10% of cancers. The VE against HPV16/18 was only 50% when we considered all vaccinated women (ITT) and just 12% when we considered persistent infections with any oncogenic HPV type, even in ATP cohorts.

For these analyses, we chose the surrogate outcome of persistent infection, which is highly reproducible (18), unlike histopathologic end points emphasized in previous reports. In previous work we have reported from Guanacaste, we compared the relative reproducibility and validity of CIN2 and CIN3 diagnoses by comparing community pathologists' diagnoses with two independent reviewers from the United States (total, $N = 357$). Two review pathologists agreed with 84% and 81%, respectively, of initial diagnoses of CIN3 compared with 13% and 31% of CIN2. Although CIN3 is a substantially more reproducible diagnosis than CIN2, the latter constitutes an important fraction of lesions in reported clinical trials (13). In addition, the virologic outcome provides direct assessment of causality in the presence of multiple infections and has a relatively high positive predictive value for subsequent development of lesions (19). The ITT analyses incorporate the reality of incomplete vaccination in mostly sexually active adults and can be extrapolated to other populations of similar age, sexual behavior, and compliance. In contrast, most women in the ATP analyses are probably naïve to HPV infection, allowing extrapolation to women vaccinated before sexual debut and who comply with vaccination regimens.

We observed statistically significant cross-protection against HPV31/33/45 as a group. There was no apparent efficacy against the very common persistent infections with HPV types other than HPV16, 18, 31, 33, and 45, an association that attenuated the overall efficacy against persistent infections with all oncogenic types down to 12%. The nominally significant deleterious effect on HPV51 may be a chance finding among many comparisons made and was not observed in the other large trials of Cervarix (20). The 4-year follow up of our study was too short to observe whether other HPV types replace vaccine types in vaccinated cohorts. Natural history data do not indicate that one HPV type modifies the epidemiology of the other (21, 22), but we did not investigate whether the presence of a nonvaccine type modifies the vaccine's protection against infection with HPV16 or HPV18.

Inclusion of women regardless of serostatus, which is imperfectly measured, allowed us to observe the full impact of the vaccine in a population, including presumably immune women. The ATP VE against HPV16 among women seronegative for HPV16 was 92.2%, approximately twice as high as that in seropositive women. The attack rate of persistent infection was lower in seropositive than seronegative women in the control arm, likely reflecting natural protection by serum antibodies and possibly other immune mechanisms (23) or reduced exposure a few years after initiation of sexual activity. The greater attack rate of persistent infection among seropositive than seronegative women in the vaccine arm may reflect high proportions of missed infections (possibly as the result of inadequate sampling of the genital tract, missed test results, or latent infections) in women who do not benefit from vaccination because they were infected before baseline. The absence of reduction in efficacy against HPV16 persistent infection among HPV18 seropositive women suggests that immune protection, rather than other correlates of sexual activity associated with antibody levels, explains the effect.

Similar efficacy against persistent infection with HPV16/18 in ATP analysis by age indicates that the vaccine is effective at protecting against new infections in unexposed women independent of age. The strong decrease in efficacy from 68% at ages 18 to 19 to 21% at ages 24 to 25 in the ITT cohort probably reflects that in the latter, there is

Table 3. VE against 1-year persistence with HPV16/18 stratified by age at enrollment

		ATP analysis[a]					ITT analysis[b]				
Age	Arm	Women, *N*	Women with events, *n*	Rate per 100 women, *n* (95% CI)	Rate reduction/ 100 women, *n* (95% CI)	VE, % (95% CI)	Women, *N*	Women with events, *n*	Rate 100 women, *n* (95% CI	Rate reduction/ 100 women, *n* (95% CI)	VE, % (95% CI)
18–19 y	Vaccine	825	1	0.1 (0.0–0.6)	2.9 (2.0–3.1)	95.9 (78.5–99.8)	1,193	28	2.3 (1.6–3.3)	5.2 (3.6–6.6)	68.9 (53.1–79.9)
	Control	870	26	3.0 (2.0–4.3)			1,244	94	7.6 (6.2–9.1)		
20–21 y	Vaccine	659	3	0.5 (0.1–1.2)	2.9 (1.6–3.6)	86.6 (59.2–96.8)	946	46	4.9 (3.6–6.4)	3.6 (1.3–5.8)	42.8 (17.9–60.6)
	Control	649	22	3.4 (2.2–5.0)			905	77	8.5 (6.8–10.5)		
22–23 y	Vaccine	588	1	0.2 (0.0–0.8)	3.8 (2.7–4.1)	95.7 (77.4–99.8)	818	36	4.4 (3.1–6.0)	4.7 (2.2–6.9)	51.5 (28.4–67.7)
	Control	625	25	4.0 (2.7–5.8)			848	77	9.1 (7.3–11.2)		
24–25 y	Vaccine	563	3	0.5 (0.1–1.4)	2.5 (1.0–3.3)	82.2 (43.9–95.9)	770	43	5.6 (4.1–7.4)	1.6 (–1.0 to 4.0)	21.8 (–16.9 to 47.9)
	Control	533	16	3.0 (1.8–4.7)			742	53	7.1 (5.5–9.2)		

[a] The ATP cohort includes women who received all 3 doses within protocol-defined windows, complied with the protocol during the vaccination period, did not have a biopsy or treatment (LEEP) before the 6-month visit, and were HPV DNA negative (by PCR) for at least one of the HPV types in the end point at enrollment and at the 6-month visit.
[b] ITT cohorts include all women randomized and vaccinated, regardless of prevalence of infection and follow-up visits.

Table 4. ATP and ITT efficacy estimates against HPV16/18 by time since first sexual intercourse at enrollment

		ATP analysis[a]					ITT analysis[b]				
Time since first sex	Arm	Women, *N*	Women with events, *n*	Rate per 100 women, *n* (95% CI)	Rate reduction/ 100 women, *n* (95% CI)	VE, % (95% CI)	Women, *N*	Women with events, *n*	Rate per 100 women, *n* (95% CI)	Rate reduction/ 100 women, *n* (95% CI)	VE, % (95% CI)
Virgin	Vaccine	566	1	0.2 (0.0–0.9)	2.6 (1.4–2.9)	93.6 (64.8–99.7)	773	4	0.5 (0.2–1.2)	2.0 (0.9–2.7)	79.8 (44.9–94.1)
	Control	615	17	2.8 (1.7–4.3)			819	21	2.6 (1.6–3.8)		
<2 y	Vaccine	227	1	0.4 (0.0–2.2)	4.5 (1.7–5.3)	91.0 (48.3–99.6)	352	12	3.4 (1.9–5.7)	7.5 (3.7–10.4)	68.7 (41.2–84.3)
	Control	244	12	4.9 (2.7–8.2)			349	38	10.9 (7.9–14.5)		
2 y	Vaccine	233	0	0.0 (0.0–1.3)	4.1 (1.8–4.1)	100.0 (62.5–100.0)	335	19	5.7 (3.6–8.6)	7.3 (2.7–11.2)	56.1 (25.2–75.0)
	Control	221	9	4.1 (2.0–7.3)			325	42	12.9 (9.6–16.9)		
3 y	Vaccine	279	0	0.0 (0.0–1.1)	5.1 (3.1–5.1)	100.0 (76.2–100.0)	395	19	4.8 (3.0–7.3)	5.8 (2.0–9.1)	54.9 (23.1–74.3)
	Control	256	13	5.1 (2.9–8.3)			394	42	10.7 (7.9–14.0)		
4+ y	Vaccine	1,330	6	0.5 (0.2–0.9)	2.4 (1.6–2.9)	84.1 (64.2–93.9)	1,872	99	5.3 (4.3–6.4)	3.2 (1.6–4.8)	38.0 (20.4–51.9)
	Control	1,341	38	2.8 (2.0–3.8)			1,852	158	8.5 (7.3–9.9)		

[a] The ATP cohort includes women who received all 3 doses within protocol-defined windows, complied with the protocol during the vaccination period, did not have a biopsy or treatment (LEEP) before the 6-month visit, and were HPV DNA negative (by PCR) for at least one of the HPV types in the end point at enrollment and at the 6-month visit.
[b] ITT cohorts include all women randomized and vaccinated, regardless of prevalence of infection and follow-up visits.

Table 5. ATP and ITT efficacy estimates against HPV16/18 by number of sexual partners at enrollment

Number of sex partners	Arm	ATP analysis[a] Women, *N*	Women with events, *n*	Rate per 100 women, *n* (95% CI)	Rate reduction/ 100 women, *n* (95% CI)	VE, % (95% CI)	ITT analysis[b] Women, *N*	Women with events, *n*	Rate per 100 women, *n* (95% CI)	Rate reduction/ 100 women, *n* (95% CI)	VE, % (95% CI)
Virgin	Vaccine	566	1	0.2 (0.0–0.9)	2.6 (1.4–2.9)	93.6 (64.8–99.7)	773	4	0.5 (0.2–1.2)	2.0 (0.9–2.7)	79.8 (44.9–94.1)
	Control	615	17	2.8 (1.7–4.3)			819	21	2.6 (1.6–3.8)		
1 partner	Vaccine	904	3	0.3 (0.1–0.9)	2.6 (1.6–3.1)	88.8 (66.5–97.3)	1,237	40	3.2 (2.4–4.3)	3.4 (1.7–4.9)	51.1 (28.9–66.7)
	Control	915	27	3.0 (2.0–4.2)			1,256	83	6.6 (5.3–8.1)		
2 partners	Vaccine	544	1	0.2 (0.0–0.9)	3.1 (1.8–3.4)	94.4 (69.1–99.7)	777	38	4.9 (3.5–6.6)	5.9 (3.1–8.3)	54.5 (33.5–69.3)
	Control	519	17	3.3 (2.0–5.1)			753	81	10.8 (8.7–13.1)		
3+ partners	Vaccine	621	3	0.5 (0.1–1.3)	4.0 (2.5–4.7)	89.2 (67.9–97.4)	940	71	7.6 (6.0–9.4)	5.2 (2.3–7.9)	40.7 (20.4–56.0)
	Control	628	28	4.5 (3.0–6.3)			911	116	12.7 (10.7–15.0)		

[a] The ATP cohort includes women who received all 3 doses within protocol-defined windows, complied with the protocol during the vaccination period, did not have a biopsy or treatment (LEEP) before the 6-month visit, and were HPV DNA negative (by PCR) for at least one of the HPV types in the end point at enrollment and at the 6-month visit.

[b] ITT cohorts include all women randomized and vaccinated, regardless of prevalence of infection and follow-up visits.

Table 6. Rates of persistent infection with HPV16/18 and VE (ATP and ITT) against HPV16/18 by time since enrollment

Time since enrollment	Arm	ATP analysis[a] Women, *N*	Women with events, *n*	Rate per 100 women, *n* (95% CI)	Rate reduction/ 100 women, *n* (95% CI)	VE, % (95% CI)	ITT analysis[b] Women, *N*	Women with events, *n*	Rate per 100 women, *n* (95% CI)	Rate reduction/ 100 women, *n* (95% CI)	VE, *n* (95% CI)
10–22 mo	Vaccine	1,599	5	0.3 (0.1–0.7)	0.8 (0.2–1.2)	71.2 (25.6–90.5)	3,056	115	3.8 (3.1–4.5)	0.7 (−0.3 to 1.7)	15.6 (−8.1 to 34.2)
	Control	1,655	18	1.1 (0.7–1.7)			3,071	137	4.5 (3.8–5.2)		
22–34 mo	Vaccine	2,190	3	0.1 (0.0–0.4)	1.6 (1.1–1.8)	91.9 (76.6–98.0)	2,870	25	0.9 (0.6–1.3)	1.3 (0.7–1.8)	59.7 (36.5–75.0)
	Control	2,239	38	1.7 (1.2–2.3)			2,913	63	2.2 (1.7–2.7)		
34–46 mo	Vaccine	1,258	0	0.0 (0.0–0.2)	1.4 (0.9–1.4)	100.0 (81.0–100.0)	3,031	11	0.4 (0.2–0.6)	1.8 (1.4–2.2)	83.5 (69.6–91.7)
	Control	1,240	17	1.4 (0.8–2.1)			3,001	66	2.2 (1.7–2.8)		
46+ mo	Vaccine	973	0	0.0 (0.0–0.3)	1.6 (1.0–1.6)	100.0 (78.6–100.0)	2,101	2	0.1 (0.0–0.3)	1.6 (1.2–1.7)	94.3 (80.1–99.1)
	Control	1,011	16	1.6 (0.9–2.5)			2,083	35	1.7 (1.2–2.3)		

[a] The ATP cohort includes women who received all 3 doses within protocol-defined windows, complied with the protocol during the vaccination period, did not have a biopsy or treatment (LEEP) before the 6-month visit, and were HPV DNA negative (by PCR) for at least one of the HPV types in the end point at enrollment and at the 6-month visit.

[b] ITT cohorts include all women randomized and vaccinated, regardless of prevalence of infection and follow-up visits.

a significantly larger fraction of women who have initiated sexual activity before vaccination and have been exposed to HPV. Rate reductions also clearly decrease with age and years since first intercourse, with the exception of virgins, who do not contribute time at risk until they start sexual activity. It should be noted, however, that the reduction in VE and rate reductions is only present in the group of women 24 to 25 years old. Interestingly, rate reductions tend to be greater among women with more partners (because their attack rate is greater) despite lower VE.

These results indicate that both susceptibility and rates of transmission are important parameters when the potential impact of prophylactic vaccines is assessed and have implications for vaccination efforts and screening policy. In the absence of a test to determine expected benefit of an individual woman, age appears to be clearly a criterion to consider for definition of target groups for vaccination. The decreases in estimates of VE seen with increasing age and time since sexual debut suggest that many infections that could eventually progress to cancer occur early and can only be prevented with adolescent vaccination.

The observation that vaccination did not substantially reduce the incidence of oncogenic infections has implications for screening programs. The positive predictive value of the tests will likely be reduced because many of the persistent infections by nonvaccine HPV types are unlikely to progress to significant lesions. The lack of reduction in infections with the lesser oncogenic types can lead to more diagnostic and therapeutic procedures than necessary in vaccinated cohorts.

Interestingly, we noted that VE against HPV16 and HPV18 tended to increase with time since vaccination, to 100% in the ATP cohort and to almost 95% in the ITT cohort, with a similar effect for the combined outcome of HPV31, 33, and 45 (to a maximum close to 60%). One possible explanation of increasing efficacy against persistent HPV16/18 infections with time since vaccination in the ATP cohort is waning influence of false-negative baseline HPV DNA results, for which efficacy is zero or low. This interpretation is supported by the reduced VE against HPV16 observed among women who were seropositive for anti-HPV16 antibodies. Similarly, the likely explanation in the ITT cohort is waning influence of baseline prevalent infections. In ITT, increased VE with time since vaccination reflects protection against new infections, but the impact of this protection in the out years needs to be interpreted in the context of the fact that exposure tends to be greatest early on after initiation of sexual activity, with reduced exposure typically observed with increasing age/time.

Most of the findings, including the stratified analyses, were similar when a 6-month HPV persistence end point was used, with the advantage that the number of end points was larger, indicating that 6-month persistence could serve as an adequate surrogate end point in HPV vaccine trials, particularly for the evaluation of HPV types that occur with lower frequency or have lower vaccine efficacies that require larger sample sizes to achieve statistical significance. In this study, for example, VE against HPV45 was not significant when the 12-month end point was used but was clearly so when the 6-month end point was used.

This analysis has some limitations and strengths. One of the limitations is that we had a relatively small sample size to accurately assess the lower efficacy of individual nonvaccine HPV types, as has been the case with other clinical trials of Cervarix (5). Those multicentric trials as well as those reported for Gardasil recruited smaller number of women in multiple research centers. In contrast, the Costa Rica HPV trial was conducted in a homogeneous population of young women at high risk of HPV infection. In this context, the results can be extrapolated to similar groups of women in areas of high HPV prevalence. It should be noted, however, that a high prevalence of HPV in young women is very common in most areas of the world, particularly those in which the vaccine is being considered to control the problem of cervical cancer. Differences in sexual practices, in particular the distribution of age at first intercourse in the population, should be taken into account when designing HPV vaccination programs.

One of the strengths of this study is that it is a large trial in a stable community, which will allow long-term follow-up up of these cohorts. Moreover, the fact that the results of this trial are very similar to those obtained in the multicentric trials points to the generalizability of VE results. The fact that participation rates at enrollment were limited could also affect the external validity of the results but not the internal validity of the randomized trial. We used virological outcomes, which have some advantages because they are highly reproducible and do not present problems for causality assessment in the presence of multiple infections. However, the clinical significance of virological outcomes, particularly for nonvaccine types, is still under active debate.

In conclusion, the clear benefit of Cervarix against persistent HPV16/18 infections observed among unexposed women decreases with age and sexual experience. These findings, together with extensive data indicating that HPV is acquired early on after sexual debut (24, 25) and the possibility of natural immunity (23), suggest limited value, in general, for vaccination beyond a few years after adolescence in areas of high prevalence of HPV infection and high risk of cervical cancer. Efforts that focus vaccination on women before sexual debut may be most effective at reaching the most vulnerable groups.

METHODS

Design, Subjects, and Procedures

The study was approved and supervised by the Institutional Review Boards of the Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA) in Costa Rica and the National Cancer Institute (NCI) in the United States. This analysis presents a double-blind randomized controlled trial of Cervarix among healthy women 18 to 25 years of age. Detailed methods have been published elsewhere (16). Potential participants from a census were invited (June 2004 to December 2005). After the participants provided informed consent, an interview, medical history, physical examination, and pregnancy test were conducted. For eligibility, women had to be healthy, not pregnant, not breastfeeding, and using contraception during the vaccination period. Main exclusion criteria were chronic diseases, history of reactions to vaccines, and history of hepatitis A or vaccination against it. Women were recruited

and randomized regardless of past sexual behavior, HPV status, or cytology.

A pelvic examination was performed on sexually experienced women. Exfoliated cells for cytology, HPV DNA, CT DNA, GC DNA, and other testing were collected with a Cervex-Brush (Rovers Medical Devices) by firmly rotating the brush 5 times 360 degrees around the cervical os. In women whose cervix exhibited extensive ectopy, the cervix brushing was also used on the ectocervix to insure sampling of the squamo-columnar junction. Blood was collected from all participants (16).

Randomization and Vaccines

Participants were randomized with equal chance to Cervarix or hepatitis A vaccine. Each dose of the HPV16/18 vaccine contained HPV16 and HPV18 L1 virus-like particle (20 μg of each) adjuvanted with 50 μg of 3-*O*-desacyl-4′-monophosphoryl lipid A and 0.5 mg of aluminum hydroxide. Each dose of the control hepatitis A vaccine contained 720 ELISA units of inactivated hepatitis A antigen and 0.5 mg of aluminum hydroxide. Both were formulated in 0.5-mL doses with identical packaging and appearance to assure blinding. Vaccination schedule consisted of 3 doses at 0, 1, and 6 months. Desirable windows for vaccination defined periods beyond which the corresponding dose was not administered (16). At 6 months, sexually active women self-collected vaginal cells for HPV testing, with results comparable with clinician-collected specimens (8).

Follow-Up

Each participant was scheduled for four annual follow-up examinations. Cytology was classified using the Bethesda system (26). Women with low-grade squamous intraepithelial lesion (LSIL) or HPV-positive atypical squamous cells of undetermined significance (ASC-US) were followed semiannually for safety until three normal results were obtained. A repeat LSIL, HPV-positive ASC-US, a single ASC-high-grade, high-grade squamous intraepithelial lesion–positive, or glandular abnormalities prompted colposcopy and treatment as needed. Unsatisfactory cytology was managed as LSIL.

Safety Monitoring

All participants were observed 30 to 60 minutes after vaccination. Adverse event and pregnancy information was actively collected during follow-up. An independent data and safety monitoring board has met regularly to examine unblinded adverse events (most recent meeting: February 2011) and repeatedly recommended continuation of the trial. The study is still blinded, and investigators had no access to unmasked data by arm; therefore, no safety data are presented in this report. However, the authors of two published reports (27, 28) on pregnancies and autoimmune conditions have included safety data from this study.

HPV DNA and Antibody Testing

Broad-spectrum PCR-based HPV DNA testing was performed at DDL Diagnostic Laboratory, on the basis of amplification and probe hybridization with use of the SPF_{10} HPV DNA enzyme immunoassay system followed by typing with the $LiPA_{25}$ version 1 line detection system as described previously (29, 30). To ensure that HPV16 and HPV18 infections were not missed, all specimens positive for HPV DNA when SPF_{10} DNA enzyme immunoassay was used but negative for HPV16 or HPV18 by $LiPA_{25}$ also were tested with type-specific primers/probes for the presence of HPV16 and HPV18 DNA (30, 31). ELISA was used for the detection and quantification of IgG antibodies against HPV16 and 18 separately by GlaxoSmithKline as described (32).

Statistical Analysis

Results presented are postlicensure analyses, conducted by an external group (Information Management Systems) under the direction of the investigators who remain masked to individuals' randomizations. We defined persistence as detection of same-type HPV in samples collected at two visits, at least 10 months apart (minimum required for two consecutive annual visits), without intervening negatives. Similarly, 6-month persistence was calculated as detection of same-type HPV in samples collected at two visits, at least 4 months apart (minimum required for two consecutive semiannual visits). There were a total of 2,668 oncogenic infections with 10+ months between first detection and last detection, of which 496 (18.6%) are not counted as persistent because of intervening negatives.

We defined different cohorts for each end point of HPV infection. ATP cohorts include women who received 3 doses within protocol-defined windows, were protocol-compliant during vaccination, had no biopsy/treatment before the 6-month visit, and were HPV DNA-negative by PCR for the corresponding HPV type at enrollment and the 6-month visit (when receiving third dose; 2,635 women in the HPV vaccine arm and 2,677 in the control arm) (16). ITT cohorts include all randomized women, regardless of compliance or enrollment infection (3,727 in the HPV arm and 3,739 in the control arm). Balance by arm overall and within subgroups was evaluated by exact binomial test when the number of women was <30 and by the analogous normal approximation to the binomial test when the total was >30.

VE is the percentage reduction in end point related to vaccine administration, estimated as the complement of the ratio of the cumulative attack rates in the HPV and control arms. The attack rate is the percentage of women in the cohort who experience the end point. The confidence interval (CI) for VE is derived from the corresponding CI for the risk ratio. The exact conditional test was used for analyses of VE. The analytical unit for all analyses is the woman rather than the infection because our principal interest is to determine the proportion of women protected against persistent HPV infections with the potential to cause cancer in the woman.

We used the difference between the attack rates in the vaccinated and control arms to address the question of absolute impact of vaccination overall and in subgroups. The CI for the difference was calculated on the basis of the exact test.

The primary objective in our prespecified plan was to evaluate VE against 1-year persistent infection with HPV16 and/or HPV18 (HPV16/18). We evaluated cross-protection against HPV31/33/45, for individual oncogenic HPV types, and for all oncogenic types combined. Six-month persistent infection also was evaluated in secondary analyses. In addition, stratified VE was calculated by enrollment covariates (age, age at first intercourse, time since first intercourse, number of sexual partners, HPV DNA, and antibody status).

Oncogenic HPV types include HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 to 73 (68–73 cannot be differentiated with genotyping method used). PCR results from all visits of a participant were included in the analyses (annual, semiannual, and colposcopy).

More than 600 women received their 3 doses outside the strict ATP windows. Separate cohorts were defined to analyze efficacy in this subgroup by the use of similar criteria for ATP and ITT as described previously.

Our results are determined on the basis of an event-triggered statistical analysis plan (SAP) approved by the U.S. Food and Drug Administration. The SAP specifies a one-sided α-level of 0.001 for

this "interim" analysis of persistent HPV16/18 infections. Results in this paper provide the most up-to-date available data from the latest data freeze of June 21, 2010. A previously published abstract for the International Papillomavirus Conference, held in July 2010, included analysis of persistent HPV16/18 infections from an earlier (January 1, 2010) data freeze; the *P*-value was $<10^{-10}$ in the ATP cohort. For regulatory purposes, we consider that 2 freezes constitute 2 separate interim analyses, leaving 0.023 ($= 0.025 - 0.001 \times 2$) as the one-sided α level when we perform our final analysis. Only the analysis of persistent HPV16/18 infections entails α spending according to the SAP. Other analyses are exploratory in the SAP and do not require adjustment.

Disclosure of Potential Conflicts of Interest

The Costa Rican Vaccine Trial is a longstanding collaboration between investigators in Costa Rica and NCI. The trial is sponsored and funded by NCI (N01-CP-11005) with support from the NIH Office of Research on Women's Health and conducted in agreement with the Ministry of Health of Costa Rica. 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. Vaccine was provided for our trial by GSK Biologicals, under a Clinical Trials Agreement with NCI. GSK also provided support for aspects of the trial associated with regulatory submission needs of the company under FDA BB-IND 7920. D.R. Lowy and J.T. Schiller are named inventors on U.S. government-owned HPV vaccine patents that are licensed to GSK and Merck, and so are entitled to limited royalties as specified by federal law. None of the other coauthors have any potential conflicts of interest to report.

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