MAJOR ARTICLE

The Impact of Quadrivalent Human Papillomavirus (HPV; Types 6, 11, 16, and 18) L1 Virus-Like Particle Vaccine on Infection and Disease Due to Oncogenic Nonvaccine HPV Types in Generally HPV-Naive Women Aged 16–26 Years

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(See the editorial commentary by Herrero and the article by Wheeler et al., on pages 919–22 and 936–44, respectively.)

Background. Human papillomavirus (HPV)–6/11/16/18 vaccine reduces the risk of HPV-6/11/16/18–related cervical intraepithelial neoplasia (CIN) 1–3 or adenocarcinoma in situ (AIS). Here, its impact on CIN1–3/AIS associated with nonvaccine oncogenic HPV types was evaluated.

Methods. We enrolled 17,622 women aged 16–26 years. All underwent cervicovaginal sampling and Pap testing at regular intervals for up to 4 years. HPV genotyping was performed for biopsy samples, and histological diagnoses were determined by a pathology panel. Analyses were conducted among subjects who were negative for 14 HPV types on day 1. Prespecified analyses included infection of ⩾6 months' duration and CIN1–3/AIS due to the 2 and 5 most common HPV types in cervical cancer after HPV types 16 and 18, as well as all tested nonvaccine types.

Results. Vaccination reduced the incidence of HPV-31/45 infection by 40.3% (95% confidence interval [CI], 13.9% to 59.0%) and of CIN1–3/AIS by 43.6% (95% CI, 12.9% to 64.1%), respectively. The reduction in HPV-31/33/45/52/58 infection and CIN1–3/AIS was 25.0% (95% CI, 5.0% to 40.9%) and 29.2% (95% CI, 8.3% to 45.5%), respectively. Efficacy for CIN2–3/AIS associated with the 10 nonvaccine HPV types was 32.5% (95% CI, 6.0% to 51.9%). Reductions were most notable for HPV-31.

Conclusions. HPV-6/11/16/18 vaccine reduced the risk of CIN2–3/AIS associated with nonvaccine types responsible for ~20% of cervical cancers. The clinical benefit of cross-protection is not expected to be fully additive to the efficacy already observed against HPV-6/11/16/18–related disease, because women may have >1 CIN lesion, each associated with a different HPV type.

Trial registration. ClinicalTrials.gov identifiers: NCT00092521, NCT00092534, and NCT00092482.

Human papillomavirus (HPV) infection is a necessary risk factor for cervical cancer [1]. Worldwide, cervical cancer is the second most common cancer in women and the third most frequent cause of death due to cancer, accounting for nearly 300,000 deaths annually [2].

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Potential conflicts of interest are listed at the end of the text.

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Table 1. Distribution of human papillomavirus (HPV) in cervical cancer (variations exist among the regions of the world).

Category	Contribution to cancer, %	Homology to HPV-16, %[a]	Homology to HPV-18, %[a]
By species			
A9 (HPV-16, -31, -33, -52, -58, and -35)	70.9	. . .	. . .
A7 (HPV-18, -45, -59, and -39)	18.6	. . .	. . .
By HPV type[b]			
HPV-16 (A9)	58.7	. . .	. . .
HPV-18 (A7)	12.2	. . .	. . .
HPV-45 (A7)	4.7	67	88
HPV-31 (A9)	3.8	83	66
HPV-33 (A9)	2.3	81	66
HPV-52 (A9)	2.2	80	66
HPV-58 (A9)	2.2	80	66
HPV-35 (A9)	1.4	82	65
HPV-59 (A7)	1.2	65	78
HPV-51 (A5)	0.7	NA	NA
HPV-56 (A6)	0.6	63	66
HPV-39 (A7)	0.5	64	77
HPV-26, -53, -66, -68, -73, and -82 (A5, A6, A7, A11)	0.9	. . .	. . .
HPV-6 and -11 (A10)	0.2	. . .	. . .
Other intermediate and low-risk types (various)	1.3	. . .	. . .
Nontypeable and infections with ⩾3 types	7.2	. . .	. . .

NOTE. For calculations of the contribution of HPV types in the context of infection with 2 or more types, a hierarchy based on the known pathogenicity of HPV types was used (16>18>31/45>52/58>33>all others). Adapted from Muñoz et al. [4]. The data-analysis plan was based on information about the distribution of HPV types in cervical cancers that was available at the time [4]. However, a recent meta-analyses suggests that, worldwide, HPV-33 is the fourth most common type in invasive carcinomas [5].

[a] Based on the evaluation of predicted amino acid sequences, using gene sequences provided by GenBank.

[b] HPV types are listed on the basis of their contribution, from highest to lowest. Species are given in parentheses.

HPV types are organized into genera and species on the basis of homology in the sequence of the L1 gene [3]. With rare exception, most of the known HPV types that infect the genital tract are members of the *Alphapapillomavirus* (or A) genus (table 1). Eighteen have been classified as oncogenic on the basis of epidemiologic and/or genetic evidence. HPV-16 is the prototype of the A9 species, which includes 6 cancer-causing types (16, 31, 33, 35, 52, and 58). HPV-18 is the prototype of the A7 species, which includes 5 cancer-causing types (18, 39, 45, 59, and 66). A pooled analysis of 11 studies conducted in 9 countries showed that the 12 most common HPV types in cervical cancer were, in descending order of frequency, 16, 18, 45, 31, 33, 52, 58, 35, 59, 51, 56, and 39 [4]. Together, HPV-16 and -18, as single infections or combined with other HPV types, are responsible for up to 70% of all invasive cervical cancers. The members of the A9 and A7 species other than HPV-16 and -18 are responsible for up to 20% of all cervical cancers and a large proportion of high- and low-grade cervical lesions [6, 7].

Recently, a quadrivalent HPV-6/11/16/18 vaccine was approved for prevention of cervical, vulvar, and vaginal intraepithelial lesions and genital warts associated with vaccine HPV types [8–11]. Given the polyclonal nature of the immune response to vaccination, it is hypothesized that anti–HPV-16 and anti–HPV-18 generated by vaccination may be able to bind to and possibly neutralize virions of HPV types closely related to 16 and/or 18, thereby preventing infection and disease associated with these other types (cross-protection). Such a capability could potentially increase the expected reduction in cancer that this vaccine will generate. Given that there is currently no established definition for cross-protection, the World Health Organization (WHO) Expert Committee on Biological Standardization recommends that demonstration of cross-protection be established by observed reductions in the incidence of cervical intraepithelial neoplasia (CIN) of any grade (abbreviated here as CIN1–3/adenocarcinoma in situ [AIS]), CIN2–3 or AIS (abbreviated here as CIN2–3/AIS) due to the types in question, and/or viral persistence, defined as detection of the same HPV type in cervicovaginal samples obtained 6, 12 or 18 months apart [12].

In the present prospective evaluation of cross-protective efficacy of the quadrivalent vaccine, we focused on 5 common HPV types whose L1 protein share at least 80% amino acid homology with the L1 protein of HPV-16 and -18 and that are individually responsible for at least 2% of cervical cancers: 31, 33, 45, 52, and 58. In addition, evaluation of cross-protection was conducted

for 5 HPV types whose homology to HPV-16 and -18 at the L1 amino acid level was <80% (35, 39, 51, 56, and 59). Together, these 10 nonvaccine HPV types account for up to 20% of all cervical cancers after excluding cases for which infection with other HPV types is present. Following the WHO guidelines, we conducted prespecified evaluations of the impact of the vaccine on the rates of infection and disease associated with these 10 nonvaccine HPV types in a population that was naive (seronegative and DNA negative) to HPV-6, -11, -16, and -18 and DNA negative to all of these 10 nonvaccine HPV types at enrollment. However, because >40 HPV types are known to infect the anogenital tract, our analysis only approximates vaccination of HPV-naive females [13]. Analyses conducted in an intention-to-treat (ITT) population including women who, before vaccination, may have been infected with 1 of the 14 HPV types of interest (approximating catch-up vaccination) are reported in the companion article by Wheeler et al. [14].

METHODS

End-point definitions. Definitions for infection and disease end points can be found in the accompanying article by Wheeler et al. [14]. The 6-month infection end point was validated using clinical trial data of the quadrivalent vaccine (see appendix A, which appears only in the electronic edition of the *Journal*).

Data sources. The analysis of disease end points used the combined database of 2 phase 3 efficacy trials: protocol 013 (NCT00092521) [8] and protocol 015 (NCT00092534) [9], termed FUTURE I and FUTURE II, respectively. Both were phase 3, randomized, double-blind, placebo-controlled clinical trials designed to investigate the prophylactic efficacy of the quadrivalent vaccine (Gardasil; Merck and Co.), as described elsewhere [8, 9]. Data for the analysis of infection end points was derived from protocol 012 (NCT00092482), a substudy of protocol 013 [15]. The studies were designed to be of 4 years' duration. Because of the high efficacy seen in FUTURE I and II, the independent data and safety monitoring board recommended vaccination of women in the placebo arm earlier than planned. The end-of-study data reported here includes ~3.6 years of post–dose 1 follow-up.

Although baseline samples were collected at enrollment, the trials allowed the enrollment of subjects who had been previously infected with or were currently infected with ≥1 vaccine HPV type or ≥1 of the 10 nonvaccine types analyzed here.

Populations. Between December 2001 and May 2003, 17,622 women aged 16–26 years (there were two 15-year-olds) were enrolled in FUTURE I ($n = 5455$, including the protocol 012 substudy [$n = 3578$]) and FUTURE II ($n = 12{,}167$). The trials enrolled women who reported having had 0–4 sex partners during their lifetime, except in Finland, where there was no such restriction. Subjects with a history of an abnormal Pap test result or treatment for genital warts (FUTURE I) were not enrolled. On day 1, subjects underwent a detailed genital examination, Pap testing, cervicovaginal sampling to detect HPV DNA, and serology for anti–HPV-6/11/16/18 testing. Subjects were randomly assigned (1:1) to receive intramuscular injections of HPV-6/11/16/18 vaccine or visually indistinguishable adjuvant-containing placebo on day 1 and at 2 and 6 months. Each protocol was approved by the institutional review boards.

Clinical follow-up and laboratory testing. ThinPrep (Cytyc) cytology samples for Pap testing were collected on day 1, at month 7, and at 6-month (FUTURE I) or 12-month (FUTURE II) intervals thereafter. Cytology samples were classified using the Bethesda System 2001 [16]. Procedures for algorithm-based cytology, colposcopy, and biopsy referral have been described elsewhere [8, 9]. Biopsy material was first read for clinical management by pathologists at a central laboratory (Diagnostic Cytology Laboratories) and then read for end-point determination by a panel of up to 4 blinded pathologists, as described elsewhere [9].

Cervical biopsy samples, endocervical curettage samples, and samples from loop electrosurgical excision procedures and conization procedures obtained at any time during the studies were tested for 14 types (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), using a polymerase chain reaction (PCR)–based assay [17–19].

The following genital swab samples were obtained at each scheduled visit: an endo/ectocervical swab (one specimen) and a combined labial/vulvar/perineal swab plus a perianal swab (pooled to become a second specimen). Ascertainment of HPV infection involved HPV PCR analysis performed on these genital swab samples. All subjects were tested for the above 14 HPV types on day 1. At months 3, 7, 12, 18, 24, 30, 36, and 48, swab samples from subjects enrolled in protocol 012 were tested for 9 HPV types (16, 18, 31, 33, 35, 45, 52, 58, and 59).

Case definition and study objectives. The primary objective was to determine whether administration of HPV-6/11/16/18 vaccine reduces the combined incidence of infection or disease associated with HPV-31 and -45 (the 2 most common types found in cervical cancer after HPV-16 and -18) and with HPV-31, -33, -45, -52, and -58 (the 5 most common HPV types found in cervical cancer after 16 and 18) [20]. Other end points were the incidence of infection or disease associated with nonvaccine A9 species members (31, 33, 35, 52, and 58), nonvaccine A7 species members (39, 45, and 59), and the 10 nonvaccine HPV types for which testing was available (31, 33, 35, 39, 45, 51, 52, 56, 58, and 59). The type(s) of HPV associated with each of the combined end points was based on genotyping HPV DNA detected in lesional tissue.

Statistical methods. Prespecified analyses were done in a population that approximates sexually naive females. This analysis was restricted to subjects who received ≥1 vaccination and, at enrollment, were seronegative and DNA negative for each of the quadrivalent HPV vaccine types (6, 11, 16, and 18); were

DNA negative for each of 10 nonvaccine types (31, 33, 35, 39, 45, 51, 52, 56, 58, and 59); and had a normal Pap test result. In this article, this population is referred to as being negative for 14 HPV types. Protocol violators were included. Case counting began after day 1.

A point estimate of vaccine efficacy (VE) and the 95% confidence interval (CI) were calculated on the basis of the observed split between vaccine and placebo recipients, adjusted for the accrued person-time in each study arm. The statistical criterion for success ($P < .05$) was equivalent to requiring that the lower bound of the CI for VE exclude 0%. An exact conditional procedure was used to evaluate VE under the assumption that the numbers of cases in the vaccine and placebo arms were independent Poisson random variables.

We evaluated VE for combined HPV types (i.e., HPV-31/45–related CIN1–3/AIS), and for individual HPV types (i.e., HPV-31–related CIN1–3/AIS). For each analysis, including those which combined multiple HPV types, a woman was counted as having an end point only once. For example, in the analysis of HPV-31/45–related disease, a woman detected with an HPV-31–related CIN2 lesion at month 12 and an HPV-45–related CIN1 lesion at month 18 would be counted once toward HPV-31/45–related CIN1–3/AIS. When VE for individual HPV types was calculated, she would count once toward HPV-31–related CIN2–3/AIS and once toward HPV-45–related CIN1–3/AIS.

Time-to-event plots shown were based on the Kaplan-Meier estimator [21]. The plots were not part of a formal survival analysis; rather, they give a visual demonstration of the divergence of incidence rates between the 2 vaccination arms over time. No direct estimation of VE should be inferred by extrapolating data from these plots.

RESULTS

FUTURE I and II enrolled 17,622 women, 99.9% of whom received ⩾1 dose of vaccine or placebo (98.4% received 2 doses and 97.2% received 3 doses). Baseline demographics for the individual studies [8, 9] and the combined studies [22] have been described elsewhere. In previously reports of VE [8, 9], the primary analysis population was per protocol. For the analyses of cross-protection, the prespecified primary analysis was done in type-specific populations that required women to be negative for the type being analyzed on day 1 only (unrestricted susceptible) rather than from day 1 through 1 month after receipt of dose 3 (per protocol) [8, 9]. Similar to the per-protocol analysis, women in the unrestricted susceptible population could be positive for other HPV types. In this primary analysis population, efficacy for HPV-31/45–related CIN1–3/AIS and high-grade lesions (CIN2–3/AIS) was 37.3% (95% CI, 17.0% to 52.8%) and 43.2% (95% CI, 12.1% to 63.9%), respectively. Efficacy for HPV-31/33/45/52/58–related CIN1–3/AIS and CIN2–3/AIS was 26.4% (95% CI, 12.9% to 37.8%) and 25.8% (95% CI, 4.6% to 42.5%), respectively. Although the primary results were favorable, the magnitude of benefit was confounded by cases of CIN2–3 associated with mixed prevalent/incident coinfection; therefore, prespecified supportive analyses were done in a population that was negative for the 14 tested HPV types, thus approximating sexually naive females. Reasons for exclusion from the efficacy analyses and baseline characteristics of the efficacy population that was negative for 14 HPV types are listed in table 2 and in figure 1. Baseline characteristics were generally well balanced between the vaccine and placebo arms. The placebo arm had a slightly higher proportion of black women (2.6% in the vaccine arm and 3.5% in the placebo arm) and virgins (9.3% in the vaccine arm and 10.4% in the placebo arm).

Subjects were followed for a mean of 3.6 years after dose 1, with 2068 (57.8% of enrolled subjects in protocol 012) included in the analyses of infection. Vaccination reduced the incidence of HPV-31/45 infection by 40.3% (95% CI, 13.9% to 59.0%) and HPV-31/33/45/52/58 infection by 25.0% (95% CI, 5.0% to 40.9%) (table 3). For individual HPV types, a significant reduction in infection was observed for HPV-31. A positive percent reduction was observed for the other 6 types analyzed, although the data did not reach statistical significance. We performed a post-hoc analysis where HPV-31 was removed from the composite end points (table 4). Efficacy for HPV-33/45/52/58 infection was 14.9% (95% CI, −11.3 to 35.0).

A total of 9296 subjects (53% of enrolled subjects in FUTURE I and II) were included in the analyses for cervical disease (tables 5 and 6). Efficacy for CIN1–3/AIS and for high-grade lesions (CIN2–3/AIS) associated with the 10 tested nonvaccine HPV types was 23.4% (95% CI, 7.8% to 36.4%) and 32.5% (95% CI, 6.0% to 51.9%), respectively. Vaccination reduced the incidence of HPV-31/45–related CIN1–3/AIS by 43.6% (95% CI, 12.9% to 64.1%) and of HPV-31/33/45/52/58–related CIN1–3/AIS by 29.2% (95% CI, 8.3% to 45.5%). Efficacy was driven primarily by reductions in HPV-31, -33, -52, and -58. No efficacy for disease was observed with respect to HPV-45, although of the 10 nonvaccine HPV types examined, HPV-45 was the least likely to be detected in CIN lesions. A post-hoc analyses where HPV-31 was removed is shown in table 4. Significant reductions in CIN1–3/AIS were observed when only the remaining 9 nonvaccine HPV types for which testing was conducted were considered.

To address any potential ascertainment bias resulting from the higher frequency of colposcopy, biopsy, and definitive therapy among placebo recipients or from the inclusion of cervical biopsy samples in the analysis of infection, we did supportive analyses whereby infection was restricted to detection of HPV DNA in cervicovaginal/anogenital swab samples only. Here, the observed efficacy for HPV-31/33/45/52/58 infection was 23.1% (95% CI, 2.1% to 39.7%).

Figure 2 shows the divergence of incidence rates over time for CIN1–3/AIS and CIN2–3/AIS for the 10 tested nonvaccine HPV

Table 2. Baseline demographics of the efficacy population that was negative for 14 human papillomavirus (HPV) types.

Characteristic	Vaccine (n = 4732)	Placebo (n = 4778)
Age, mean ± SD, years	19.8 ± 2.1	19.8 ± 2.1
Race		
Asian	215/4732 (4.5)	225/4778 (4.7)
Black	122/4732 (2.6)	166/4778 (3.5)
Hispanic American	550/4732 (11.6)	521/4778 (10.9)
Native American	6/4732 (0.1)	3/4778 (0.1)
White	3,437/4732 (72.6)	3443/4778 (72.1)
Other	402/4732 (8.5)	420/4778 (8.8)
Lifetime no. of sex partners at enrollment		
0 (virgin)	441/4732 (9.3)	495/4778 (10.4)
1	1913/4732 (40.4)	1899/4778 (39.7)
2	1124/4732 (23.8)	1107/4778 (23.2)
3	747/4732 (15.8)	734/4778 (15.4)
4	473/4732 (10.0)	498/4778 (10.4)
⩾4	34/4732 (0.7)	42/4778 (0.9)
Prevalence of *Chlamydia trachomatis*	99/4651 (2.1)	92/4679 (2.0)
Prevalence of *Neisseria gonorrhoeae*	15/3389 (0.4)	5/3404 (0.1)
Past pregnancy	885/4732 (18.7)	879/4778 (18.4)

NOTE. Data are no. (%) of subjects, unless otherwise indicated. This population was restricted to subjects who received ⩾1 vaccination and, at enrollment, were seronegative and DNA negative for each of the quadrivalent HPV vaccine types (6, 11, 16, and 18); were DNA negative for each of 10 nonvaccine types (31, 33, 35, 39, 45, 51, 52, 56, 58, and 59); and had a normal Pap test result. Selected baseline demographics of the overall population can be found in reference 22. Percentages were calculated as (no. of subjects with indicated characteristic/no. of subjects with a known response or satisfactory test result) × 100.

types. Over the course of follow-up, reductions in the incidence of disease became increasingly apparent for the 10 tested nonvaccine HPV types. As shown in figure 2, the censoring of subjects who reach an end point markedly decreases the sample size; therefore, the 95% CIs for the incidence rates grow wider with longer follow-up. Direct estimation of VE cannot be inferred by extrapolating data from these plots. Efficacy (tables 3–6) was calculated on the basis of a fixed-event design with the analysis performed at a single point in time at the end of follow-up, with all subjects in the analysis population providing data.

The cross-protective efficacy was most apparent and consistent for members of the A9 species. The combined incidence of HPV-31/33/35/52/58–related CIN1–3/AIS was reduced by 31.9% (95% CI, 11.8% to 47.6%). For the A7 species, a positive percent reduction was observed with respect to HPV-39– and HPV-59–related end points, although the reductions were not statistically significant. There was no evidence for efficacy with respect to HPV-51–related CIN (A5 species). A positive percent reduction (not statistically significant) was observed for HPV-56–related CIN (A6 species).

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

Figure 1. Reasons for exclusion from the efficacy population that was negative for 14 human papillomavirus (HPV) types (infection and disease end points).

Efficacy findings for CIN1–3/AIS were consistent between the individual protocols, with the exception of HPV-35–, HPV-51–, and HPV-59–related end points, for which nonsignificant reductions were observed in protocol 013 but not protocol 015 (data not shown).

Although subjects were required to be DNA negative for all 14 HPV types on day 1 in order to be included in the efficacy evaluations, the presence of ⩾1 type in incident CIN lesions was common, consistent with prior natural history studies. A total of 308 incident CIN2–3/AIS lesions were observed in the placebo arm during the follow-up period (note: a women may have developed more than one lesion during the course of the studies, but for each row in the analyses of VE, each woman was counted only once). Of the 308 incident CIN2–3/AIS lesions observed in the placebo arm, 138 (44.8%) were associated with HPV-16–related A9 species, with 99 (32.1%) being associated with HPV-31/33/35/52 and/or 58 with no coinfection with vaccine HPV types and with 39 (12.7%) being associated with HPV-31/33/35/52 and/or 58 with coinfection with vaccine HPV types. In the vaccine arm, lesions associated with a mix of vaccine and nonvaccine HPV types were rare, because the prophylactic efficacy against disease due to vaccine HPV types approaches 100% [8, 9].

Table 3. Analysis of cross-protection against infection in the efficacy population that was negative for 14 human papillomavirus (HPV) types (prespecified analyses).

Category	Vaccine (n = 1036)		Placebo (n = 1032)		Efficacy (95% CI), %
	Cases	Rate[a]	Cases	Rate[a]	
HPV-31 or -45	49	1.4	81	2.3	40.3 (13.9 to 59.0)
HPV-31, -33, -45, -52, or -58	127	3.8	167	5.0	25.0 (5.0 to 40.9)
Individual HPV types[b]					
HPV-31	31	0.9	57	1.6	46.2 (15.3 to 66.4)
HPV-33	15	0.4	21	0.6	28.7 (−45.1 to 65.8)
HPV-45	24	0.7	26	0.7	7.8 (−67.0 to 49.3)
HPV-52	50	1.4	61	1.7	18.4 (−20.6 to 45.0)
HPV-58	35	1.0	37	1.0	5.5 (−54.3 to 42.2)
Other HPV types tested[b]					
HPV-35	14	0.4	17	0.5	17.8 (−77.1 to 62.5)
HPV-59	45	1.3	55	1.6	18.7 (−22.8 to 46.4)
Nonvaccine A9 species (HPV-31, -33, -35, -52, and -58)	124	3.7	157	4.7	21.9 (0.6 to 38.8)
Nonvaccine A7 species (HPV-45 and -59)	66	1.9	77	2.2	14.8 (−19.9 to 39.6)

NOTE. This population was restricted to subjects who received ⩾1 vaccination and, at enrollment, were seronegative and DNA negative for each of the quadrivalent HPV vaccine types (6, 11, 16, and 18); were DNA negative for each of 10 nonvaccine types (31, 33, 35, 39, 45, 51, 52, 56, 58, and 59); and had a normal Pap test result. Infection is defined as detection of the same HPV type on 2 consecutive visits spaced ⩾6 months apart (±1-month visit windows) or the presence of cervical/genital disease associated with the relevant type (with DNA for the type found in a swab sample at the visit directly before or after the biopsy). A subject is counted only once within each applicable row. CI, confidence interval.

[a] Cases per 100 person-years at risk.

[b] The study was not powered to assess efficacy against individual types.

DISCUSSION

HPV types other than 6, 11, 16, and 18 that cause a substantial proportion of cervical HPV disease, including cancer, are structurally related to HPV-16 and -18. It is important to examine the prophylactic efficacy of the HPV-6/11/16/18 vaccine in preventing the acquisition of infection and related cervical disease associated with these nonvaccine types in populations approximating those targeted by most HPV vaccination programs. Thus, the present study evaluated the cross-protective efficacy of the vaccine in a subset of the clinical trial population that, before vaccination, was negative for the 14 HPV types tested and had a normal Pap test result, approximating sexually naive females. This allowed for an assessment of cross-protective efficacy in the absence of prevalent infections. We observed a 32.5% reduction in CIN2–3/AIS associated with 10 nonvaccine HPV types that collectively cause ~20% of cervical cancers. These findings are the first demonstration of cross-protection for any HPV vaccine against CIN2–3/AIS, disease end points that are cervical cancer precursors and that formed the basis of vaccine licensure. Reductions of similar magnitudes were observed for the combined end points of CIN1–3/AIS. The cross-protective efficacy was driven by the A9 species members, particularly HPV-31, -33, -52, and -58.

Although we observed significant reductions in cervical disease due to HPV types not included in the vaccine, the clinical benefit of cross-protection (in terms of numbers of lesions prevented) should not be expected to be fully additive to the efficacy already observed against HPV-6/11/16/18–related disease, because many women have >1 CIN lesion and each lesion may be associated with a different HPV type. For example, among placebo subjects, the cumulative incidence of CIN2–3/AIS for the A9 species (HPV-31, -33, -35, -52, and -58) was 14.7 events per 1000 subjects. Of the 69 cases in the placebo arm, 22 (31.9%) occurred in women who also had an HPV-16– or HPV-18–related CIN2–3/AIS lesion. Had these women been immunized before exposure, the HPV-16/18 disease would have been prevented. In the absence of cross-protection, the prevention of HPV-16/18 infection would not have ended their risk of developing CIN2–3 due to a nonvaccine type. However, with our observed cross-protection, the risk of CIN2–3 due to a nonvaccine HPV type was reduced. As shown in figure 3, the added benefit of cross-protection resulted in the prevention of an additional 3 cases of CIN2–3, an increment of 4.3%. Despite an observed benefit, as with any vaccine the long-term reductions in the overall burden of disease—including reductions in Pap testing and abnormalities, colposcopy, and definitive therapy—have yet to be determined.

Table 4. Analysis of cross-protection against infection and disease excluding human papillomavirus (HPV)–31 (post-hoc analyses).

The table is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

Table 5. Analysis of cross-protection against cervical intraepithelial neoplasia (CIN) 1–3 or adenocarcinoma in situ (AIS) due to human papillomavirus (HPV) types other than 16 and 18 in the efficacy population that was negative for 14 HPV types (prespecified analyses).

Category	Vaccine (n = 4616)		Placebo (n = 4680)		Efficacy (95% CI), %
	Cases	Rate[a]	Cases	Rate[a]	
HPV-31 or -45	34	0.2	61	0.4	43.6 (12.9 to 64.1)
HPV-31, -33, -45, -52, or -58	103	0.6	147	0.9	29.2 (8.3 to 45.5)
HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, or -59	205	1.3	270	1.6	23.4 (7.8 to 36.4)
Nonvaccine A9 species (HPV-31, -33, -35, -52, or -58)[b]	101	0.6	150	0.9	31.9 (11.8 to 47.6)
HPV-31	23	0.1	54	0.3	56.9 (28.6 to 74.8)
HPV-33	18	0.1	30	0.2	39.2 (−12.6 to 68.1)
HPV-35	11	0.1	14	0.1	20.4 (−88.8 to 67.3)
HPV-52	35	0.2	51	0.3	30.6 (−8.9 to 56.2)
HPV-58	36	0.2	44	0.3	17.1 (−31.7 to 48.2)
Nonvaccine A7 species (HPV-39, -45, or -59)[b]	54	0.3	75	0.5	27.3 (−4.6 to 49.7)
HPV-39	28	0.2	42	0.3	32.6 (−11.4 to 59.8)
HPV-45	11	0.1	10	0.1	−11.3 (−192.2 to 57.1)
HPV-59	20	0.1	26	0.2	22.3 (−44.7 to 58.9)
HPV-51[b]	66	0.4	64	0.4	−4.3 (−49.5 to 27.2)
HPV-56[b]	58	0.4	81	0.5	27.6 (−2.6 to 49.3)

NOTE. This population was restricted to subjects who received ⩾1 vaccination and, at enrollment, were seronegative and DNA negative for each of the quadrivalent HPV vaccine types (6, 11, 16, and 18); were DNA negative for each of 10 nonvaccine types (31, 33, 35, 39, 45, 51, 52, 56, 58, and 59); and had a normal Pap test result. Disease was defined as the diagnosis of a tissue sample as CIN, AIS, or cervical cancer by a pathology panel with DNA detected in tissue from the same lesion. A subject is counted only once within each applicable row. CI, confidence interval.

[a] Cases per 100 person-years at risk.

[b] The study was not powered to assess efficacy against individual types.

The WHO recommends that demonstration of cross-protection be established by observed reductions in CIN and/or viral persistence. There is currently no international consensus on a definition for HPV persistence based on type-specific detection of HPV DNA by PCR [12]. We considered 6-month infection for the same HPV genotype. Single-time detection of HPV was not an end point, because a single positive HPV DNA test result may be due to transient infection, contamination, or early persistent infection. Previous reports of a bivalent HPV vaccine showed some level of cross-protection against infection with HPV-45, -31, and -52; however, disease end points were not reported [23]. Efficacy analyses using disease end points rather than infection alone are necessary to measure the clinical benefit of cross-protection.

In the present study, when excluding the vaccine types, the cumulative incidence of CIN1–3/AIS through 3.6 years of follow-up was highest for HPV-56, -51, -31, -52, and -58, in descending order. Lesions associated with HPV-45 were rare. We observed reductions in the incidence of CIN lesions associated with A9 species members, particularly HPV-31. It is likely that second-generation HPV vaccines targeting a broader spectrum of oncogenic HPV types may be available within the next decade and would include those HPV types that cause the highest proportion of cervical cancers. Given that some of these types, such as HPV-56, contribute to <1% of the worldwide cervical cancer burden, it is noteworthy to observe reductions in HPV types that may not be included in second-generation vaccines.

The present study has some limitations. Our combined end points included lesions with strong malignant potential (CIN3/AIS). However, the study was not designed with sufficient power to detect reductions in CIN2–3/AIS due to nonvaccine types or to measure reductions in individual nonvaccine HPV types, explaining why a posteriori there is not sufficient power to measure these reductions. Our analyses also required that women be DNA negative for nonvaccine types on day 1 only; thus, some women may have become infected before receiving all 3 doses. In addition, subjects in the placebo arm were more likely to be referred for colposcopic examination, biopsy, and definitive therapy, because of the lack of protection from HPV-6/11/16/18 infection. This could lead to potential ascertainment bias. It was therefore important to corroborate the observed reductions in cervical disease associated with nonvaccine types with infection data from swab samples only, which would not be subject to this potential bias.

Because women remain at risk for HPV infection as long as they remain sexually active, it will be important to determine the duration of protection against HPV-related disease for both vaccine and nonvaccine types. It has not been possible to establish a minimum protective antibody titer for vaccine types; however, the quadrivalent vaccine elicits immune memory, a hallmark of long-term pro-

Table 6. Analysis of cross-protection for cervical intraepithelial neoplasia (CIN) 2–3 or adenocarcinoma in situ (AIS) due to human papillomavirus (HPV) types other than 16 and 18 in the efficacy population that was negative for 14 HPV types (prespecified analyses).

HPV type	Vaccine (n = 4616)		Placebo (n = 4680)		Efficacy (95% CI), %
	Cases	Rate[a]	Cases	Rate[a]	
HPV-31 or -45	11	0.1	27	0.2	58.7 (14.1 to 81.5)
HPV-31, -33, -45, -52, or -58	44	0.3	66	0.4	32.5 (−0.3 to 55.0)
HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, or -59	62	0.4	93	0.6	32.5 (6.0 to 51.9)
Nonvaccine A9 species (HPV-31, -33, -35, -52, or -58)[b]	44	0.3	69	0.4	35.4 (4.4 to 56.8)
HPV-31	8	<0.1	27	0.2	70.0 (32.1 to 88.2)
HPV-33	12	0.1	16	0.1	24.0 (−71.2 to 67.2)
HPV-35	4	<0.1	4	<0.1	−1.5 (−444.9 to 81.1)
HPV-52	17	0.1	23	0.1	25.2 (−46.4 to 62.5)
HPV-58	16	0.1	20	0.1	18.9 (−64.7 to 60.7)
Nonvaccine A7 species (HPV-39, -45, or -59)[b]	11	0.1	21	0.1	47.0 (−15.0 to 76.9)
HPV-39	4	<0.1	10	<0.1	59.6 (−40.2 to 90.7)
HPV-45	3	<0.1	2	<0.1	−51.9 (−1717.8 to 82.6)
HPV-59	5	<0.1	9	<0.1	43.8 (−86.9 to 85.2)
HPV-51[b]	16	0.1	15	0.1	−8.1 (−134.7 to 50.0)
HPV-56[b]	12	0.1	16	0.1	24.1 (−71.1 to 67.2)

NOTE. This population was restricted to subjects who received ⩾1 vaccination and, at enrollment, were seronegative and DNA negative for each of the quadrivalent HPV vaccine types (6, 11, 16, and 18); were DNA negative for each of 10 nonvaccine types (31, 33, 35, 39, 45, 51, 52, 56, 58, and 59); and had a normal Pap test result. Disease was defined as the diagnosis of a tissue sample as CIN, AIS, or cervical cancer by a pathology panel with DNA detected in tissue from the same lesion. A subject is counted only once within each applicable row. CI, confidence interval.

[a] Cases per 100 person-years at risk.

[b] The study was not powered to assess efficacy against individual types.

tection [24]. Follow-up of large cohorts will be required to establish the duration of efficacy of the vaccine for both vaccine and nonvaccine types.

The efficacy of the vaccine among 16–26-year-old women with a lifetime history of ⩾4 sex partners was not evaluated. Although the present analysis focused on cross-protection in a population that was representative of an HPV-naive population, an ITT analysis of all women entering the FUTURE I and II trials regardless of baseline HPV status also demonstrated statistically significant cross-protective reductions in both HPV infections and CIN1 or greater due to nonvaccine oncogenic HPV types [14].

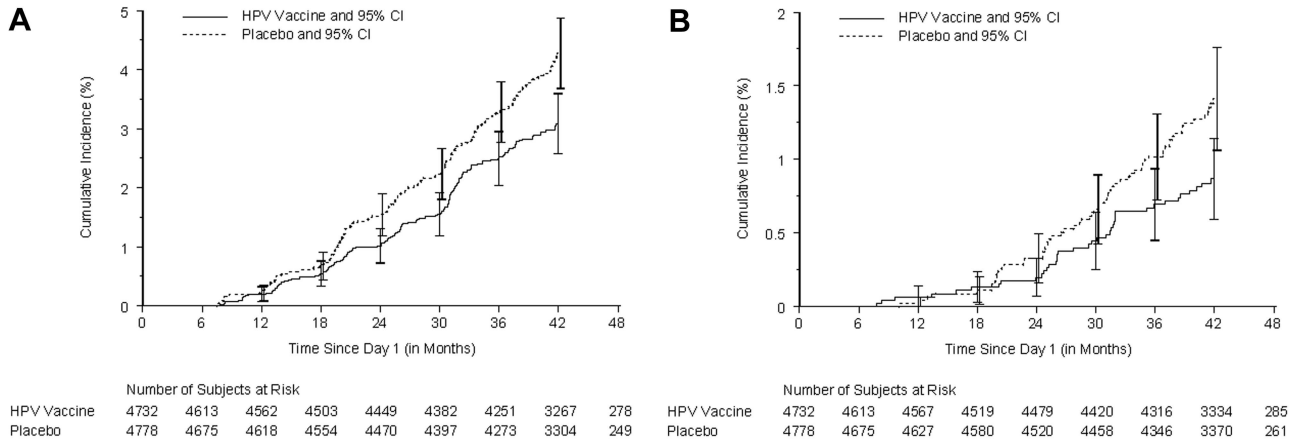

Figure 2. Time to detection of human papillomavirus (HPV)–31/33/35/39/45/51/52/56/58/59–related cervical intraepithelial neoplasia (CIN) 1–3 or adenocarcinoma in situ (AIS) *(A)* and HPV-31/33/35/39/45/51/52/56/58/59–related CIN2–3/AIS *(B)* in the efficacy population that was negative for 14 HPV types. This population was restricted to subjects who received ⩾1 vaccination and, at enrollment, were seronegative and DNA negative for each of the quadrivalent HPV vaccine types (6, 11, 16, and 18); were DNA negative for each of 10 nonvaccine types (31, 33, 35, 39, 45, 51, 52, 56, 58, and 59); and had a normal Pap test result. CI, confidence interval.

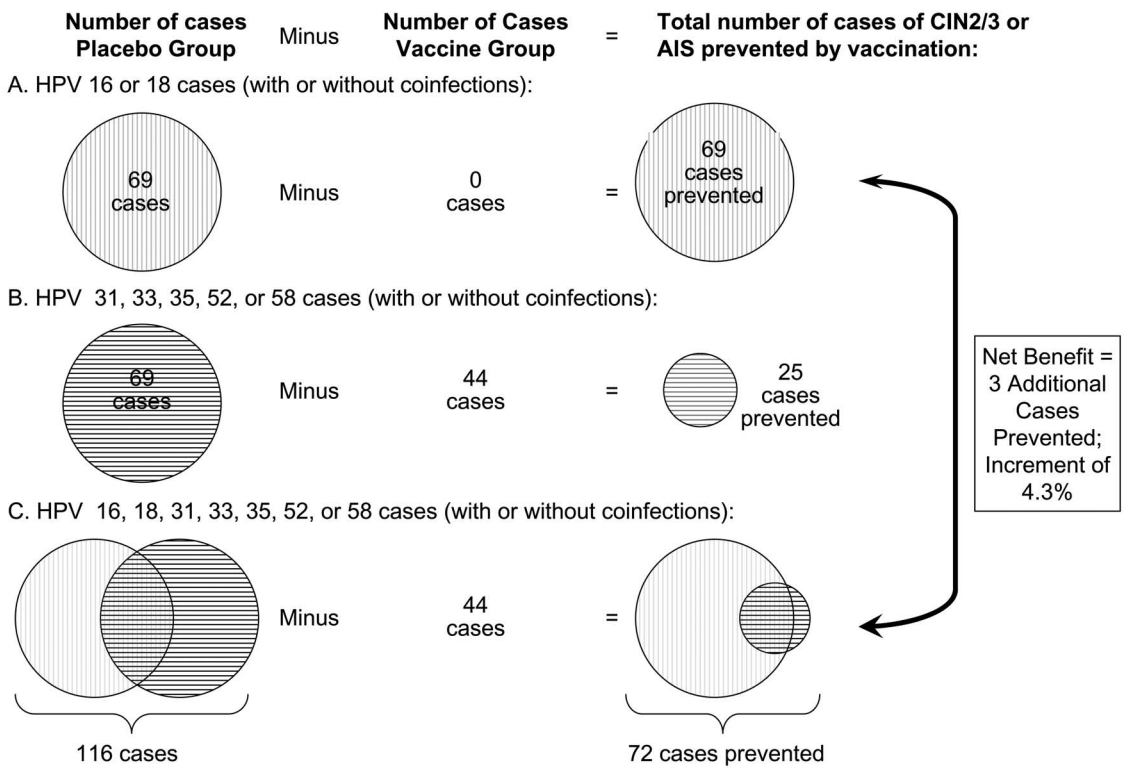

Figure 3. Illustration of the added benefit of cross-protection. AIS, adenocarcinoma in situ; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus.

In conclusion, the results of the HPV-6/11/16/18 vaccine program provide strong evidence that implementation of HPV vaccination campaigns in HPV-naive preadolescent girls and young adult women has the potential to reduce cervical cancer rates worldwide. The demonstrated cross-protection against additional oncogenic HPV types may provide an extra measure of protection for young women immunized with the quadrivalent HPV vaccine.

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APPENDIX A

PREDICTIVE VALUE OF DURATION OF INFECTION

We assessed the predictive value of the duration of infection as it relates to subsequent progression to CIN2–3/AIS. Subjects from the placebo arm of protocol 012 who received ⩾1 vaccination and who were seronegative and DNA negative for the relevant HPV type on day 1 were included in the analysis. Subjects were considered to have developed an end point if either of the following occurred: (1) 1 or more episodes of infection related to a particular HPV type occurred without the subsequent development of CIN2–3/AIS related to the same HPV type (in this instance, subjects were classified according to length of the episode that had the longest duration); (2) infection related to a particular HPV type occurred with subsequent diagnosis of CIN2–3/AIS related to the same type. Subjects were classified according to the duration of infection immediately preceding the diagnosis of CIN2–3/AIS.

Table A1. Summary of subjects with human papillomavirus (HPV) infection from the placebo arm of protocol 012 (unrestricted susceptible population) who developed cervical intraepithelial neoplasia (CIN) 2 or worse related to the same HPV type, by duration of infection.

HPV type, duration of infection	Subjects with subsequent diagnosis of CIN2–3/AIS related to the indicated HPV type[a]	Subjects without subsequent diagnosis of CIN2–3/AIS related to the indicated HPV type[b]	PPV, point estimate (95% CI), %[c]
HPV-16			
<6 months	6	38	13.6 (5.2 to 27.4)
⩾6 to <12 months	10	59	14.5 (7.2 to 25.0)
⩾12 months	8	82	8.9 (3.9 to 16.8)
HPV-18			
<6 months	3	22	12.0 (2.5 to 31.2)
⩾6 to <12 months	1	26	3.7 (0.1 to 19.0)
⩾12 months	1	31	3.1 (0.1 to 16.2)
HPV-31			
<6 months	5	24	17.2 (5.8 to 35.8)
⩾6 to <12 months	4	37	9.8 (2.7 to 23.1)
⩾12 months	3	49	5.8 (1.2 to 15.9)
HPV-33			
<6 months	1	9	10.0 (0.3 to 44.5)
⩾6 to <12 months	3	10	23.1 (5.0 to 53.8)
⩾12 months	3	19	13.6 (2.9 to 34.9)
HPV-45			
<6 months	0	17	0
⩾6 to <12 months	0	16	0
⩾12 months	0	23	0
HPV-52			
<6 months	4	20	16.7 (4.7 to 37.4)
⩾6 months to 12 months	2	43	4.4 (0.5 to 15.1)
⩾12 months	3	50	5.7 (1.2 to 15.7)
HPV-58			
<6 months	3	21	12.5 (2.7 to 32.4)
⩾6 to <12 months	1	31	3.1 (0.1 to 16.2)
⩾12 months	0	26	0

NOTE. Includes all subjects who received ⩾1 vaccination and were seronegative and DNA negative for the relevant HPV type(s) on day 1. Cases were counted starting 30 days after day 1. AIS, adenocarcinoma in situ; CI, confidence interval; PPV, positive predictive value.

[a] No. of subjects with infection related to the indicated HPV type for the duration specified in each row who subsequently developed a case of CIN2–3/AIS related to the indicated HPV type. Subjects are classified according to the duration of infection related to the indicated HPV type immediately preceding the diagnosis of CIN2–3/AIS related to the indicated HPV type.

[b] Subjects who developed 1 or more episodes of infection related to the indicated HPV type without developing a case of CIN2–3/AIS related to the indicated HPV type. Subjects are classified in the row that corresponds to the episode of infection with the longest duration.

[c] Percentages were calculated as (no. of subjects with subsequent CIN2–3/AIS related to the same HPV type/total no. of subjects with infection related to the indicated HPV type) × 100.

The total number of subjects who had an infection end point was computed as the sum of the number of subjects who reached either of the 2 end points. The positive predictive value of duration of infection as it relates to subsequent progression to CIN2–3/AIS was defined as the proportion of the total subjects who developed a case of infection who subsequently developed a case of CIN2–3/AIS. The point estimate of the positive predictive value was calculated as (no. of subjects with subsequent CIN2–3/AIS related to the same HPV type/total no. of subjects with infection related to the indicated HPV type) $\times$ 100. The 95% CI was calculated following the usual derivation of a 95% CI for the point estimate of the probability p from a binomial distribution.

Table A1 shows the number of cases of infection classified according to duration of infection, along with the positive predictive value of duration of infection as it relates to subsequent progression to CIN2–3/AIS. In summary, the observed positive predictive value of infection that is $<$12 months' duration (i.e., $>$0 to $<$6 months or $\geq$6 to $<$12 months) was higher than that of an infection that is $\geq$12 months' duration.

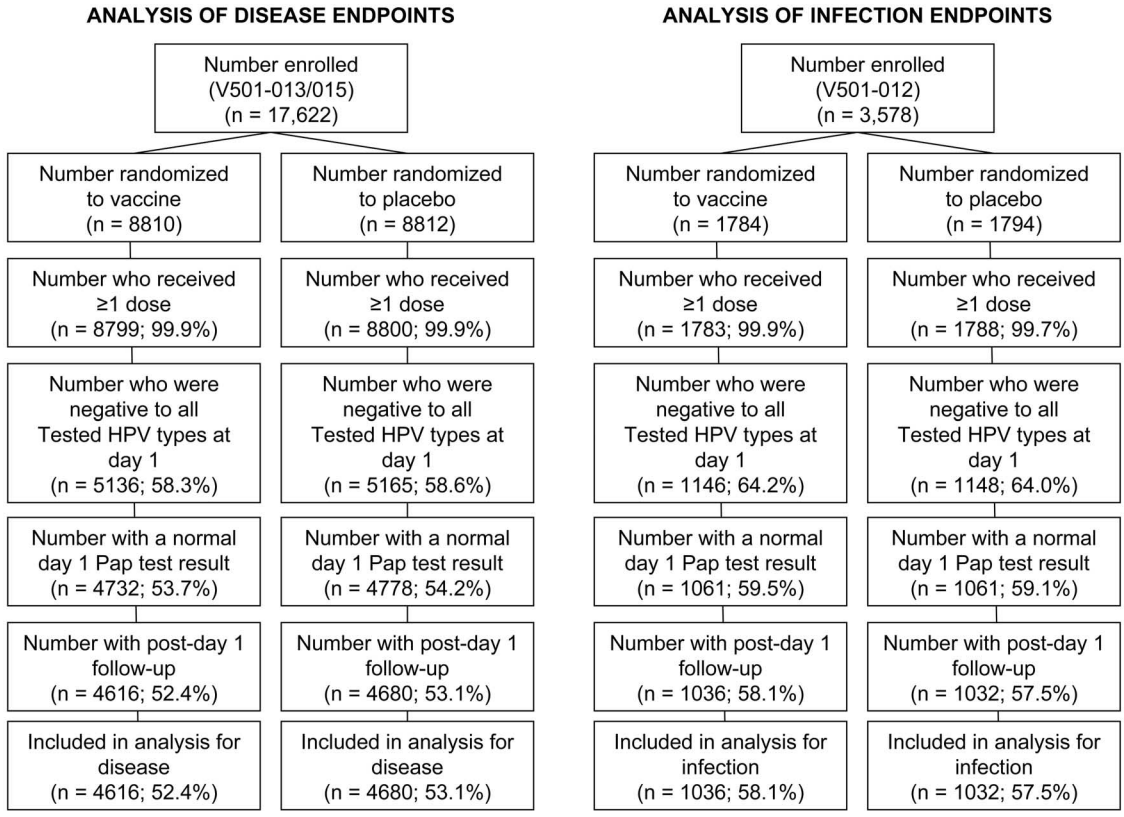

Figure 1. Reasons for exclusion from the efficacy population that was negative for 14 human papillomavirus (HPV) types (infection and disease end points).

Table 4. Analysis of cross-protection against infection and disease excluding human papillomavirus (HPV)–31 (post-hoc analyses).

Category	Vaccine			Placebo			Efficacy (95% CI), %
	No.	Cases	Rate[a]	No.	Cases	Rate[a]	
Infection[b]							
HPV-33, -45, -52, or -58	1036	105	3.1	1032	123	3.6	14.9 (−11.3 to 35.0)
Nonvaccine A9 species (HPV-33, -35, -52, or -58)	1036	100	2.9	1032	114	3.4	12.5 (−15.5 to 33.8)
CIN1–3/AIS							
HPV-33, -45, -52, or -58	4616	89	0.5	4680	114	0.7	21.0 (−5.1 to 40.8)
Nonvaccine A9 species (HPV-33, -35, -52, or -58)	4616	87	0.5	4680	117	0.7	24.8 (−0.1 to 43.7)
HPV-33, -35, -39, -45, -51, -52, -56, -58, or -59	4616	196	1.2	4680	251	1.5	21.2 (4.6 to 34.9)
CIN2–3/AIS							
HPV-33, -45, -52, or -58	4616	40	0.2	4680	50	0.3	19.0 (−25.3 to 47.9)
Nonvaccine A9 species (HPV-33, -35, -52, or -58)	4616	40	0.2	4680	53	0.3	23.5 (−17.5 to 50.6)
HPV-33, -35, -39, -45, -51, -52, -56, -58, or -59	4616	59	0.4	4680	83	0.5	28.0 (−1.7 to 49.4)

NOTE. A subject is counted only once within each applicable row. AIS, adenocarcinoma in situ; CI, confidence interval; CIN, cervical intraepithelial neoplasia.

[a] Cases per 100 person-years at risk.

[b] Defined as detection of the same HPV type on 2 consecutive visits spaced ⩾6 months apart (±1-month visit windows) or the presence of cervical/genital disease associated with the relevant type (with DNA for the type found in a swab sample at the visit directly before or after the biopsy).