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Quadrivalent Vaccine against Human Papillomavirus to Prevent High-Grade Cervical Lesions

The FUTURE II Study Group*

ABSTRACT

BACKGROUND

Human papillomavirus types 16 (HPV-16) and 18 (HPV-18) cause approximately 70% of cervical cancers worldwide. A phase 3 trial was conducted to evaluate a quadrivalent vaccine against HPV types 6, 11, 16, and 18 (HPV-6/11/16/18) for the prevention of high-grade cervical lesions associated with HPV-16 and HPV-18.

METHODS

In this randomized, double-blind trial, we assigned 12,167 women between the ages of 15 and 26 years to receive three doses of either HPV-6/11/16/18 vaccine or placebo, administered at day 1, month 2, and month 6. The primary analysis was performed for a per-protocol susceptible population that included 5305 women in the vaccine group and 5260 in the placebo group who had no virologic evidence of infection with HPV-16 or HPV-18 through 1 month after the third dose (month 7). The primary composite end point was cervical intraepithelial neoplasia grade 2 or 3, adenocarcinoma in situ, or cervical cancer related to HPV-16 or HPV-18.

RESULTS

Subjects were followed for an average of 3 years after receiving the first dose of vaccine or placebo. Vaccine efficacy for the prevention of the primary composite end point was 98% (95.89% confidence interval [CI], 86 to 100) in the per-protocol susceptible population and 44% (95% CI, 26 to 58) in an intention-to-treat population of all women who had undergone randomization (those with or without previous infection). The estimated vaccine efficacy against all high-grade cervical lesions, regardless of causal HPV type, in this intention-to-treat population was 17% (95% CI, 1 to 31).

CONCLUSIONS

In young women who had not been previously infected with HPV-16 or HPV-18, those in the vaccine group had a significantly lower occurrence of high-grade cervical intraepithelial neoplasia related to HPV-16 or HPV-18 than did those in the placebo group. (ClinicalTrials.gov number, NCT00092534.)

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Cervical cancer is the second most common cancer in women and the leading cause of cancer-related death in many developing countries.[1] Although well-organized programs for Papanicolaou screening have led to a significant decline in mortality from cervical cancer in developed countries,[1] such programs are costly[2] and have not been effectively implemented in most developing countries.[3]

Human papillomaviruses (HPVs) cause virtually all cervical cancers, with HPV types 16 (HPV-16) and 18 (HPV-18) responsible for approximately 70%.[4] When phase 3 trials of prophylactic HPV vaccines were in the planning stages, the vaccine advisory committee of the Food and Drug Administration (FDA) recommended that these trials be powered to demonstrate efficacy in preventing high-grade cervical lesions that are classified as cervical intraepithelial neoplasia grade 2 or 3,[5] a perspective endorsed by the World Health Organization (WHO).[6] In this report, we describe the results of a phase 3 trial of a quadrivalent vaccine against HPV types 6, 11, 16, and 18 (HPV-6/11/16/18) designed to assess the prevention of cervical intraepithelial neoplasia grade 2 or 3, adenocarcinoma in situ, and cervical cancer caused by HPV-16 or HPV-18. HPV-6 and HPV-11, which are rarely detected in high-grade cervical lesions, cause the majority of anogenital warts.[7,8] A report by Garland et al.[9] on vaccine efficacy against external anogenital, vaginal, and cervical lesions associated with HPV types 6, 11, 16, and 18 appears elsewhere in this issue of the *Journal*.

Methods

Study Design

From June 2002 through May 2003, we enrolled 12,167 women between the ages of 15 and 26 years at 90 study sites in 13 countries in our ongoing double-blind, placebo-controlled, randomized trial. The institutional review board at each center approved the protocol; written informed consent was obtained from all subjects. Women were eligible to participate in the study if they were not pregnant, did not report abnormal results on a Papanicolaou smear, and had had a lifetime number of no more than four sex partners. Subjects were asked to use effective contraception during the vaccination period (day 1 through month 7).

The trial, which was called Females United to Unilaterally Reduce Endo/Ectocervical Disease (FUTURE) II, was designed, managed, and analyzed by Merck in conjunction with external academic investigators and members of the external data and safety monitoring board. The academic authors had full access to the data and the analyses and approved the final manuscript. All authors vouch for the completeness and accuracy of the data presented.

Vaccine and Randomization

The quadrivalent HPV-6/11/16/18 virus-like–particle vaccine with amorphous aluminum hydroxyphosphate sulfate adjuvant (Gardasil, Merck) and a visually indistinguishable aluminum-containing placebo have been described previously.[10] Subjects were randomly assigned to receive vaccine or placebo at day 1, month 2, and month 6 after having a negative result on a pregnancy test of the urine or blood. (Details regarding the randomization procedure can be found in the Supplementary Appendix, available with the full text of this article at www.nejm.org.) Subjects were observed for 30 minutes after receiving the injection and were asked to report serious adverse events occurring 1 to 15 days after each injection. A total of 916 subjects (all of the subjects at U.S. centers) were asked to use a vaccination report card to record all serious and nonserious adverse events occurring 1 to 15 days after each injection. Throughout the trial, all serious adverse events that were potentially related to either the procedure or the vaccine, all deaths, and all pregnancy outcomes were to be reported.

Clinical Follow-up

After randomization, the first-day visit included a gynecologic examination and the taking of a medical history with collection of cervical samples for Papanicolaou testing (ThinPrep, Cytyc) and anogenital swabs (of the labial, vulvar, perineal, perianal, endocervical, and ectocervical areas) for HPV DNA testing. Follow-up visits were scheduled 1 and 6 months after the third injection and at months 24, 36, and 48. (Details regarding specimen collection and HPV testing can be found in the Supplementary Appendix.)

Cervical Lesions

Referrals for colposcopy were standardized with the use of a mandatory Papanicolaou triage algorithm (Fig. 1 of the Supplementary Appendix). Colposcopists were trained to locate and biopsy all discrete abnormal areas on the cervix. Separate instruments were used to avoid HPV contamina-

tion. Biopsy samples were processed and adjacent histologic sections of each sample were first read for clinical management by pathologists at a central laboratory (Diagnostic Cytology Laboratories) who were unaware of treatment-group assignments and HPV status and then read for end-point determination by a panel of four pathologists who were unaware of diagnoses made at the central laboratory, clinical findings, treatment group, and HPV status. Subjects with cervical intraepithelial neoplasia with a severity of at least grade 2 or 3 were referred for definitive therapy.

PRIMARY HYPOTHESIS AND END POINTS

The primary hypothesis stated that, as compared with placebo, the vaccine would reduce the incidence of high-grade cervical intraepithelial neoplasia related to HPV-16 or HPV-18 in the per-protocol susceptible population. End-point assignment was based on the blinded consensus diagnosis of at least two pathologists. The primary composite end point was cervical intraepithelial neoplasia grade 2 or 3, adenocarcinoma in situ, or invasive carcinoma of the cervix, with the detection of DNA from HPV-16, HPV-18, or both in one or more of three adjacent sections of the same lesion.[9] The evaluations were performed with the use of WHO histologic criteria.[11,12]

STATISTICAL ANALYSIS

The study was powered on the basis of a fixed number of events with an interim analysis. To ensure adequate power for the interim analysis (a power of 80 to 90%, with a one-sided alpha of 0.0102) and the final analysis (a power of at least 90%, with a one-sided alpha of 0.02055) for true vaccine efficacy of 80 to 90%, at least 19 subjects with the primary composite end point were required for the interim analysis; at least 29 subjects with the primary composite end point were required for the final analysis. With an anticipated annual event rate of 0.19% for HPV-16–related end points and 0.038% for HPV-18–related end points, a total of 11,500 subjects were required for the study. The interim analysis for vaccine efficacy (approximately 1.5 years of follow-up after administration of the third dose, including all data from visits that occurred before June 11, 2005) showed 100% efficacy against high-grade cervical intraepithelial neoplasia related to HPV-16 or HPV-18 in the per-protocol population, with no subjects in the vaccine group and 21 in the placebo group (97.96% confidence interval [CI], 76 to 100). This analysis was part of the application for vaccine licensure, which was approved after priority review by the FDA on June 8, 2006. The analyses presented here include an additional year of follow-up with all data from visits that occurred on or before June 15, 2006.

The prespecified primary efficacy analysis (per-protocol analysis) was conducted among subjects who had negative results on DNA and serologic testing for HPV-16 or HPV-18 at enrollment, remained DNA-negative for the same HPV type through 1 month after the administration of the third dose of vaccine or placebo (month 7), received all doses within 1 year, and had no protocol violations. Follow-up for case ascertainment for this analysis started 1 month after the administration of the third dose of vaccine or placebo. The primary hypothesis was tested at the one-sided alpha level of 0.02055 with the use of a multiplicity adjustment[13] to account for the interim analysis. A point estimate of vaccine efficacy and the 95.89% CI were calculated on the basis of the observed split between subjects receiving vaccine and those receiving placebo and the accrued person-time. The statistical criterion for success ($P<0.02055$) was equivalent to requiring that the lower bound of the 95.89% CI for vaccine efficacy would exclude 0%. An exact conditional procedure was used to evaluate vaccine efficacy under the assumption that the numbers of patients with high-grade cervical disease in the vaccine and placebo groups were independent Poisson random variables.[14] For subjects who had more than one end-point event, only the first event in a category was counted as a case (defined as a consensus diagnosis), but a subject with more than one end-point event could be counted in more than one category of end-point events.

Analyses were conducted with respect to the primary end point, which was further characterized by lesion type and by positivity for HPV-16, HPV-18, or both. A woman with a lesion containing both HPV-16 and HPV-18 DNA or with different lesions showing various histologic grades was counted only once toward the primary composite end point and once for each of the component end points defined on the basis of HPV-16 or HPV-18 status or lesion grade.

To estimate vaccine efficacy in a population with less than perfect compliance, a prespecified supportive analysis was conducted in an unrestricted susceptible population that included all subjects who had negative results on polymerase-

chain-reaction (PCR) and serologic assays to the relevant type of HPV at enrollment. We also estimated vaccine efficacy in an intention-to-treat population that included all subjects who had undergone randomization, regardless of baseline status with respect to HPV and cervical neoplasia. Follow-up for case ascertainment in these populations started 1 day after the first injection. The intention-to-treat population was used to evaluate the effect of vaccination on prevalent and incident HPV-16– or HPV-18–related high-grade disease and high-grade disease caused by either vaccine or nonvaccine HPV types. For the intention-to-treat-populations, cumulative incidence distributions[15] for each vaccination group were computed and presented graphically with 95% CIs.

Subjects in the per-protocol susceptible population from whom serum samples were collected during predefined time frames were included in type-specific immunogenicity analyses. Adverse events were summarized for all vaccination visits as frequencies and percentages according to study group. Risk differences and associated 95% CIs (unadjusted for multiplicity) were com-

Table 1. Baseline Characteristics of the Subjects.*

Characteristic	Vaccine Group (N = 6087)	Placebo Group (N = 6080)
General		
Mean age — yr	20.0±2.2	19.9±2.1
Region — no./total no. (%)		
Asia–Pacific	92/6087 (1.5)	89/6080 (1.5)
North America	460/6087 (7.6)	456/6080 (7.5)
Latin America	1599/6087 (26.3)	1594/6080 (26.2)
Europe	3936/6087 (64.7)	3941/6080 (64.8)
Sexual and gynecologic history		
Sexual activity among nonvirgins†		
Mean age at first sexual intercourse — yr	16.6±1.9	16.6±1.9
Median lifetime no. of sex partners	2	2
Past pregnancy — no./total no. (%)	1242/6085 (20.4)	1218/6079 (20.0)
Use of hormonal contraception — no./total no. (%)	3613/6082 (59.4)	3614/6075 (59.5)
Baseline HPV-associated pathological finding		
Positive results on HPV-16 testing — no./total no. (%)		
DNA detection by PCR	543/5997 (9.1)	545/6008 (9.1)
Serologic analysis	652/6066 (10.7)	688/6065 (11.3)
Positive results on HPV-18 testing — no./total no. (%)		
DNA detection by PCR	230/6011 (3.8)	242/6013 (4.0)
Serologic analysis	227/6065 (3.7)	236/6064 (3.9)
Chlamydia trachomatis–positive at day 1 — no./total no. (%)	258/5981 (4.3)	224/5961 (3.8)
Cytologic abnormality present at day 1 — no./total no. (%)	697/5919 (11.8)	654/5896 (11.1)
Atypical squamous cells		
Undetermined significance	280/5919 (4.7)	274/5896 (4.6)
Cannot exclude high-grade squamous intraepithelial lesion	21/5919 (0.4)	18/5896 (0.3)
Squamous intraepithelial lesion		
Low-grade	352/5919 (5.9)	326/5896 (5.5)
High-grade	42/5919 (0.7)	33/5896 (0.6)
Atypical glandular cells	2/5919 (<0.1)	3/5896 (<0.1)

* Plus–minus values are means ±SD. PCR denotes polymerase chain reaction.
† Ninety-three percent of subjects were nonvirgins.

puted comparing the vaccine group with the placebo group.

RESULTS

A total of 12,707 women attended the enrollment visit. Of these, 12,167 (96%) met the eligibility requirements; of the eligible subjects, 6087 were randomly assigned to receive vaccine and 6080 to receive placebo. Baseline characteristics were similarly distributed between the two groups (Table 1). At baseline, the results of Papanicolaou tests were abnormal for 11.8% of subjects in the vaccine group and 11.1% in the placebo group (Table 1). The most common reason for exclusion from the two prespecified populations for analysis of prophylactic efficacy was PCR-based detection of HPV-16 or HPV-18 DNA or antibodies at baseline (Tables 1 and 2).

In this ongoing study, subjects were followed for an average of 3 years after the administration of the first dose of vaccine or placebo. In the per-protocol susceptible population, which included 10,565 of 12,167 women who underwent random-

Table 2. Subjects Included in and Excluded from the Analyses.*

Variable	Vaccine Group (N=6087)	Placebo Group (N=6080)
	no. (%)	
Subjects included in analyses		
Per-protocol susceptible population		
At risk for HPV-16	4559 (74.9)	4408 (72.5)
At risk for HPV-18	5055 (83.0)	4970 (81.7)
Unrestricted susceptible population		
At risk for HPV-16	5054 (83.0)	5043 (82.9)
At risk for HPV-18	5602 (92.0)	5602 (92.1)
Intention-to-treat population	6087 (100)	6080 (100)
Reason for exclusion from analyses†		
Per-protocol and unrestricted susceptible populations		
Seropositive or PCR positive to HPV-16 at day 1‡§	948 (15.6)	962 (15.8)
Seropositive or PCR positive to HPV-18 at day 1‡§	397 (6.5)	405 (6.7)
Missing day 1 serologic samples or results	9 (0.1)	8 (0.1)
Day 1 serologic sample out of acceptable day range	8 (0.1)	3 (<0.1)
Missing day 1 swab samples or results	109 (1.8)	82 (1.3)
Day 1 swab sample out of acceptable day range	3 (<0.1)	2 (<0.1)
Per-protocol susceptible population only		
General protocol violations¶	275 (4.5)	316 (5.2)
Missed dose 2 or 3 of vaccine or placebo	128 (2.1)	99 (1.6)
Missing month 7 swab samples or results‖	159 (2.6)	136 (2.2)
Seropositive or PCR positive to HPV-16 at or before month 7 (inclusive)‡§	1005 (16.5)	1160 (19.1)
Seropositive or PCR positive to HPV-18 at or before month 7 (inclusive)‡§	441 (7.2)	549 (9.0)

* PCR denotes polymerase chain reaction.
† Subjects may have been excluded for more than one reason.
‡ The exclusion criterion applies only to the analysis populations for the respective HPV type.
§ The exclusion criterion applies to either seropositivity or PCR positivity on day 1 but to only PCR positivity at or before month 7.
¶ The most common general protocol violations were obtaining the month 7 swab outside the acceptable range of days (204 subjects) and the administration of immunosuppressive drugs, immunoglobulin G, or blood products (122 subjects). Swab specimens obtained more than 14 days before or 10 days after administration of the first dose of vaccine or placebo were considered to be unacceptable.
‖ The exclusion criterion applies to subjects who received all three vaccinations.

Table 3. Vaccine Efficacy against Cervical Intraepithelial Neoplasia Grade 2 or 3 or Adenocarcinoma In Situ Associated with HPV-16 or HPV-18 or Any HPV Type.*

End Point	Vaccine Group (N=6087)			Placebo Group (N=6080)			Vaccine Efficacy
	Total Subjects	No. of Cases	Rate†	Total Subjects	No. of Cases	Rate†	
							% (95% CI)‡
Lesions associated with HPV-16 or HPV-18							
Subjects in per-protocol susceptible population	5305	1	<0.1	5260	42§	0.3	98 (86–100)¶
Lesion type							
Cervical intraepithelial neoplasia grade 2	5305	0	0	5260	28	0.2	100 (86–100)
Cervical intraepithelial neoplasia grade 3	5305	1	<0.1	5260	29	0.2	97 (79–100)
Adenocarcinoma in situ	5305	0	0	5260	1	<0.1	100 (<0–100)
HPV type							
HPV-16	4559	1	<0.1	4408	35	0.3	97 (84–100)
HPV-18	5055	0	0	4970	11	0.1	100 (61–100)
Subjects in unrestricted susceptible population‖	5865	3	<0.1	5863	62**	0.4	95 (85–99)
Lesion type							
Cervical intraepithelial neoplasia grade 2	5865	1	<0.1	5863	40	0.2	97 (85–100)
Cervical intraepithelial neoplasia grade 3	5865	2	<0.1	5863	43	0.3	95 (82–99)
Adenocarcinoma in situ	5865	0	0	5863	4	<0.1	100 (<0–100)
HPV type							
HPV-16	5054	3	<0.1	5043	51	0.3	94 (82–99)
HPV-18	5602	0	0	5602	16	0.1	100 (74–100)
Subjects in intention-to-treat population††	6087	83	0.5	6080	148	0.8	44 (26–58)
Lesion type							
Cervical intraepithelial neoplasia grade 2	6087	41	0.2	6080	96	0.5	57 (38–71)
Cervical intraepithelial neoplasia grade 3	6087	57	0.3	6080	104	0.6	45 (23–61)
Adenocarcinoma in situ	6087	5	<0.1	6080	7	<0.1	28 (<0–82)
HPV type							
HPV-16	6087	77	0.4	6080	132	0.8	42 (22–56)
HPV-18	6087	6	<0.1	6080	29	0.2	79 (49–93)

ization (87%), the vaccine prevented 98% of HPV-16/18–related high-grade cervical lesions (Table 3). In this population, 1 woman in the vaccine group and 42 women in the placebo group received the diagnosis of cervical intraepithelial neoplasia grade 2 or 3 or cervical adenocarcinoma in situ associated with HPV-16, HPV-18, or both. The single subject whose disease was counted as a case (defined as a consensus diagnosis) of HPV-16–positive cervical intraepithelial neoplasia grade 3 in the vaccine group was positive for HPV-52 at baseline as well as in five histologic specimens collected at the time of diagnosis and treatment. HPV-16 DNA was detected in one histologic specimen but at no other time points.

A total of 11,508 of 12,167 women who under-

Table 3. (Continued.)

End Point	Vaccine Group (N=6087)			Placebo Group (N=6080)			Vaccine Efficacy
	Total Subjects	No. of Cases	Rate†	Total Subjects	No. of Cases	Rate†	
							% *(95% CI)*‡
Lesions associated with any HPV type							
Subjects in intention-to-treat population	6087	219	1.3	6080	266	1.5	17 (1–31)
Lesion type							
Cervical intraepithelial neoplasia grade 2	6087	149	0.9	6080	192	1.1	22 (3–38)
Cervical intraepithelial neoplasia grade 3	6087	127	0.7	6080	161	0.9	21 (<0–38)
Adenocarcinoma in situ	6087	5	<0.1	6080	8	<0.1	37 (<0–84)

* Subjects were counted only once within each applicable row. Some subjects were counted in more than one row. Subjects in the per-protocol population tested negative for HPV-16 and HPV-18 on polymerase chain reaction (PCR) and were seronegative to the relevant HPV type at enrollment, remained PCR-negative for the same HPV type through 1 month after administration of the third dose, received three doses of vaccine or placebo within 1 year, and did not violate the protocol. Subjects in the unrestricted susceptible population tested negative for HPV-16 or HPV-18 on PCR and serologic analysis at enrollment. Subjects in the intention-to-treat population included all subjects who had undergone randomization, including those with prevalent cervical disease, HPV-16 or HPV-18 infections, and infections caused by other high-risk HPV types before vaccination.

† The rate is the number of subjects with the end point per 100 person-years at risk.

‡ The confidence interval (CI) is 95% for all intervals except for the first row in the per-protocol susceptible population, for which the CI is 95.89%, reflecting a multiplicity adjustment for the primary efficacy analysis.

§ In the per-protocol susceptible population, the worst histologic diagnoses in the 42 subjects in the placebo group were cervical intraepithelial neoplasia grade 2 (12 subjects), cervical intraepithelial neoplasia grade 3 (29), and adenocarcinoma in situ (1).

¶ P<0.001.

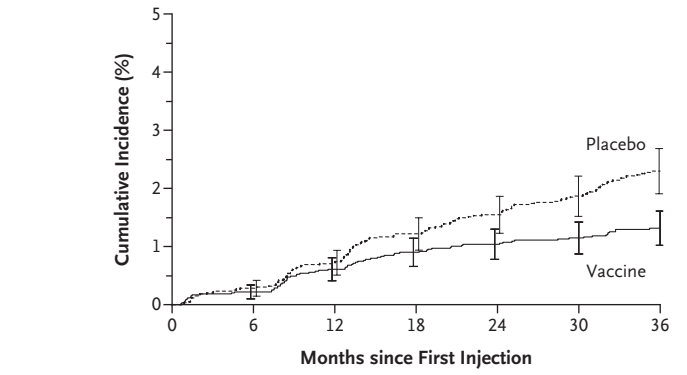
‖ Of subjects in the unrestricted susceptible population who received at least one dose of vaccine or placebo and had at least one follow-up visit after administration of the first dose, 5739 in the vaccine group and 5769 in the placebo group were included in the analysis for HPV-16 and HPV-18 end points, 4952 and 4961, respectively, were included in the analysis for HPV-16 end points, and 5480 and 5511, respectively, were included in the analysis for HPV-18 end points.

** In the unrestricted susceptible population, the worst histologic diagnoses in the 62 subjects in the placebo group were cervical intraepithelial neoplasia grade 2 (16 subjects), cervical intraepithelial neoplasia grade 3 (42), and adenocarcinoma in situ (4).

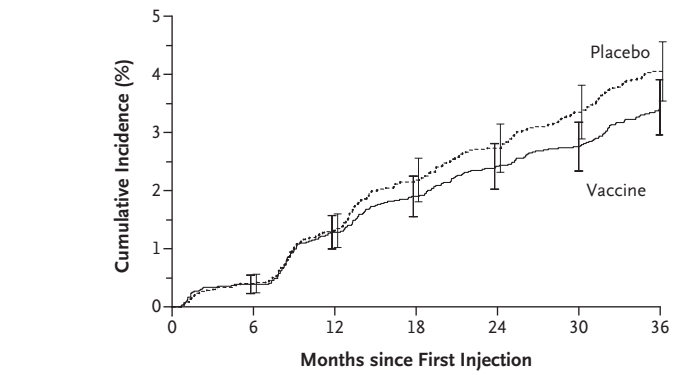
†† In the intention-to-treat population, 5951 subjects in the vaccine group and 5977 in the placebo group received at least one vaccine dose and had at least one follow-up visit after the first dose. The intention-to-treat analysis included an analysis of all biopsy specimens regardless of the reason the procedure was performed and all biopsy specimens that were performed outside the context of the study.

went randomization (95%) were included in the analysis of the unrestricted susceptible population. Vaccine efficacy remained high at 95% (Table 3). Cervical intraepithelial neoplasia grade 2 or 3 or adenocarcinoma in situ developed in 3 subjects in the vaccine group and in 62 in the placebo group. Of note, more than 99% of subjects in this population eventually received the full three-dose regimen.

To provide a preliminary assessment of the effect of quadrivalent vaccine on high-grade cervical disease related to HPV-16 or HPV-18 in a population that included women with and without prevalent cervical intraepithelial neoplasia and infection due to vaccine and nonvaccine HPV types at baseline, we performed an intention-to-treat analysis of all women who had undergone randomization (Fig. 1A and Table 3). Vaccine efficacy was 44%, with high-grade cervical disease related to HPV-16 or HPV-18 developing in 83 subjects in the vaccine group and 148 in the placebo group. Most of the cases (defined as consensus diagnoses) that were added to the first intention-to-treat analysis (98%), as compared with those in the unrestricted susceptible population, were high-grade cervical disease caused by HPV-16 or HPV-18 infection that was present before the first injection. Vaccination did not appear to alter the course of cervical lesions related to HPV-16 or HPV-18 or of infection present at the time of randomization (Table 1 of the Supplementary Appendix). Thus, since the percentage of subjects with an end point associated with infection or disease that was prevalent at baseline decreased over time, the incidence of HPV-16–related or HPV-18–related cervical intraepithelial neoplasia



No. at Risk							
Vaccine	6087	5918	5824	5733	5592	5427	2925
Placebo	6080	5942	5832	5736	5586	5420	2994
Cumulative No. of Subjects with an End Point							
Vaccine	0	13	36	53	61	67	76
Placebo	0	17	43	72	91	109	132



No. at Risk							
Vaccine	6087	5915	5808	5709	5564	5406	2909
Placebo	6080	5939	5819	5718	5569	5399	2982
Cumulative No. of Subjects with an End Point							
Vaccine	0	23	76	112	142	161	196
Placebo	0	24	78	129	161	196	234

Figure 1. Time until the Development of High-Grade Cervical Disease Associated with HPV-16, HPV-18, or Any HPV Type (Intention-to-Treat Population).

The numbers include all subjects who underwent randomization, including those who had prevalent cervical disease, infection with HPV-16 or HPV-18, or infection with other high-risk HPV types before vaccination. Panel A shows the cumulative incidence of cervical intraepithelial neoplasia grade 2 or 3 or adenocarcinoma in situ associated with HPV-16 or HPV-18 (the composite primary end point). Panel B shows the cumulative incidence of cervical intraepithelial neoplasia grade 2 or 3 or adenocarcinoma in situ associated with any HPV type. In the intention-to-treat population, 5951 in the vaccine group and 5977 in the placebo group received at least one dose of vaccine and had at least one follow-up visit after the first dose. The graph terminates at 36 months because only a small number of subjects were at risk after 36 months. I bars represent 95% confidence intervals.

grade 2 or 3 and adenocarcinoma in situ in the placebo group continued to increase while the incidence in the vaccine group began to plateau (Fig. 1A). In the second intention-to-treat analysis, 219 subjects in the vaccine group and 266 in the placebo group had prevalent or incident high-grade cervical disease due to vaccine or nonvaccine HPV types, representing a reduction of 17% in the vaccine group (Fig. 1B and Table 3). None of the women had invasive cervical cancer.

Among women who tested negative for both HPV-16 and HPV-18 at enrollment (4693 in the vaccine group and 4703 in the placebo group), high-grade cervical disease developed in 95 subjects in the vaccine group and in 130 in the placebo group, a reduction of nearly 27% in the vaccine group (95% CI, 4 to 44). Women who were infected with only HPV-16 or HPV-18 appeared to be protected against disease caused by the type of HPV to which they had tested negative. Though the result was not significant, vaccine efficacy for women who tested positive for HPV-16 (773 in the vaccine group and 798 in the placebo group) appeared to be high for cervical intraepithelial neoplasia grade 2 or 3 and adenocarcinoma in situ caused by HPV-18 (no subjects in the vaccine group and 5 in the placebo group). Vaccine efficacy for women who tested positive for HPV-18 (257 subjects in the vaccine group and 253 in the placebo group) also appeared to be high for cervical intraepithelial neoplasia grade 2 or 3 and adenocarcinoma in situ caused by HPV-16 (no subjects in the vaccine group and 2 in the placebo group).

Among the 1512 vaccinated women in the immunogenicity substudy, more than 99% had seroconversion to the relevant vaccine-type HPV. At month 24, of those subjects in each type-specific per-protocol population included in the immunogenicity substudy, 96% of 986 subjects were seropositive for HPV-6, 97% of 987 were seropositive for HPV-11, 99% of 953 were seropositive for HPV-16, and 68% of 1059 were seropositive for HPV-18, as measured in specific neutralizing antibodies. Although the percentage of women in the vaccine group who maintained detectable levels of HPV-18 antibodies was lower than the percentage with HPV-16 antibodies, the efficacy for prevention of HPV-18–related high-grade lesions was maintained at 100% through follow-up in the unrestricted susceptible population (Table 3).

There were relatively few side effects of vaccination. The proportion of subjects who reported one or more injection-site adverse events was higher in the vaccine group than in the placebo group (84.4% vs. 77.9%), with the most common event being injection-site pain (risk difference, 6.5 percentage points; 95% CI, 1.4 to 11.7) (Table 4). One subject in the placebo group discontinued participation owing to a serious injection-related adverse event (hypersensitivity). The proportions of women reporting serious adverse events were similar in the two treatment groups. (All systemic and serious adverse events, categorized by organ system and treatment group, are provided in Tables 5 and 6 of the Supplementary Appendix.) Within these categories, there were nominally significant differences in the percentages of subjects in the vaccine group and the placebo group who reported seasonal allergies (10 in the vaccine group and 2 in the placebo group [risk difference, 1.8; 95% CI, 0.3 to 3.7]) and neck pain (2 in the vaccine group and 10 in the placebo group [risk difference, −1.8; 95% CI, −3.7 to −0.3]). No multiplicity adjustments were made for these comparisons. Adverse-event profiles were generally similar for women with and without antibodies to one or more of the vaccine-related HPV types at enrollment.

Pregnancy was reported in 1053 subjects in the vaccine group and 1106 in the placebo group. For all phase 3 trials of the quadrivalent vaccine combined (Table 2 of the Supplementary Appendix), 1396 subjects in the vaccine group (13%) and 1436 in the placebo group (16%) had become pregnant, and outcomes were available for approximately 82%. The proportions of women with live births, difficulties with delivery, spontaneous abortions, and late fetal deaths were similar in the two study groups. Congenital anomalies were reported for 41 infants and 6 fetuses (25 in the vaccine group and 22 in the placebo group) (Table 3 of the Supplementary Appendix). A review by an external specialist who was unaware of study-group assignments concluded that the types of anomalies observed were diverse and consistent with those generally seen in young women.[16]

Further analyses were done to evaluate pregnancies with an estimated date of conception either within 30 days after any vaccination or more than 30 days after any vaccination (Table 4 of the Supplementary Appendix). For pregnancies with an estimated date of conception within 30 days after any vaccination, 19 of 112 in the vaccine group and 26 of 115 in the placebo group resulted in a spontaneous abortion (risk difference, −5.6; 95% CI, −16.1 to 4.8). Of the live-born infants, five were found to have a congenital anomaly (five in the vaccine group and none in the placebo group [risk difference, 4.5; 95% CI, 1.1 to 10.1]). The congenital anomalies observed in these five infants were relatively common and pathogenetically unrelated, suggesting different causes. For pregnancies with an estimated date of conception of more than 30 days after any vaccination, 266 of 1198 in the vaccine group and 283 of 1218 in the placebo group resulted in a spontaneous abortion (risk difference, −1.0; 95% CI, −4.4 to 2.3). A total of 20 infants or fetuses whose mothers were in the vaccine group and 22 whose mothers were in the placebo group were found to have a congenital anomaly (risk difference, −0.1; 95% CI, −1.2 to 1.0).

DISCUSSION

When administered to subjects who had not been previously exposed to either HPV-16 or HPV-18, the prophylactic HPV vaccine was highly effective (98%) in preventing HPV-16–related and HPV-18–related cervical intraepithelial neoplasia grade 2 or 3 and adenocarcinoma in situ. Efficacy was lower (44%) for the population of all women who had undergone randomization, which also included subjects who had HPV-16–related or HPV-18–related cervical intraepithelial neoplasia or infection with HPV-16 or HPV-18 before the first injection. Although prevention of invasive cervical cancer is the main goal of prophylactic HPV vaccination, it is ethically unacceptable to use invasive cancer as the end point in efficacy trials. Cervical intraepithelial neoplasia grade 3 and adenocarcinoma in situ,[12] which the International Federation of Obstetrics and Gynecology classifies as stage 0 noninvasive cervical cancers,[17] are clinically important outcomes because they are likely to persist[18-20] and may become invasive without treatment. Although a histologic diagnosis of cervical intraepithelial neoplasia grade 2 is less reproducible and spontaneous regression is more common than for grade 3,[19,20] this lesion is also considered to be high grade.[12]

We took several steps to enhance accuracy, reproducibility, and generalizability of our findings. To ensure high sensitivity for histologic end

Table 4. Adverse Events.*

Variable	Vaccine Group	Placebo Group	Difference in Risk (95% CI)
Any adverse event among subjects in safety cohort†			
No. of subjects who received ≥1 injection	457	454	
No. of subjects with follow-up data	448	447	
Subjects with ≥1 events — no. (%)			
Injection-site event	378 (84.4)	348 (77.9)	6.5 (1.4 to 11.7)
Pain	372 (83.0)	339 (75.8)	7.2 (1.9 to 12.5)
Systemic event	275 (61.4)	268 (60.0)	1.4 (–5.0 to 7.8)
Serious adverse event among all subjects‡			
No. of subjects with follow-up data	6019	6031	
Subjects with event — no. (%)			
Any serious event	45 (0.7)	54 (0.9)	–0.1 (–0.5 to 0.2)
Serious injection-related event§	3 (<0.1)	2 (<0.1)	0 (–0.1 to 0.1)
Discontinuation — no. (%)			
Serious adverse event	7 (0.1)	6 (0.1)	0 (–0.1 to 0.2)
Serious injection-related event	0	1 (<0.1)	0 (–0.1 to 0.1)
Death¶	7 (0.1)	5 (0.1)	0 (–0.1 to 0.2)

* The difference in risk is the number in the vaccine group minus the number in the placebo group. A 95% CI that does not include zero indicates a statistically significant difference at a two-sided alpha level of 0.05. No multiplicity adjustments were made for these comparisons.

† The safety cohort includes subjects who completed the vaccination report card from day 1 through day 15 after each vaccination. Of the 916 subjects in the safety cohort, 913 received at least one dose of vaccine or placebo. Two subjects received a mixed regimen of clinical material and were excluded from the summary.

‡ This category includes all subjects with safety follow-up data.

§ In the vaccine group, the serious adverse events were gastroenteritis, headache, hypertension, injection-site pain, and a decrease in joint movement at the injection site. In the placebo group, the serious adverse events were hypersensitivity to the injection (for which one subject discontinued participation in the study), chills, headache, and fever.

¶ Causes of death in the vaccine group were pneumonia and sepsis, overdose of an illicit drug, traffic accident (three subjects), pulmonary embolism, and infective thrombosis. The causes of death in the placebo group were suicide (two subjects), asphyxia, and traffic accident (two subjects). None of the deaths were judged by the research investigator to be related to vaccine or placebo.

points, colposcopists were instructed to biopsy all discrete abnormal areas; for high specificity, HPV-16 and HPV-18 DNA had to be detected in tissue sections. Histologic diagnoses were determined by a panel of expert gynecologic pathologists who were unaware of other clinical and laboratory data. Generalizability was enhanced by enrolling women from both developed and developing nations and by using standard Papanicolaou screening and management algorithms.[21,22] Prophylactic vaccine efficacy was high for all four geographic regions and for all ethnic or racial groups and was similar to efficacy estimates from phase 2 trials of HPV-16 and HPV-18 infection,[10,23] indicating the robustness of our findings.

The mean length of follow-up in this study was 3 years after administration of the first dose of vaccine or placebo. Within this interval, 42 subjects in the placebo group who were included in the per-protocol analysis became infected, and incident high-grade cervical disease related to HPV-16 or HPV-18 developed. As others have reported,[24-28] the time from HPV infection to the development of a high-grade cervical lesion is often less than 24 months. These findings suggest that widespread immunization of female adolescents and young women could lead to reductions in HPV-16–related and HPV-18–related high-grade lesions that would be apparent within years, rather than decades.

Although follow-up was relatively short in duration, three previous phase 2 trials of prophylactic HPV recombinant vaccines included 4 to 5 years of follow-up[28-30] and found no evidence of waning immunity or decreased efficacy for prevention of infection or persistent shedding of virus. Durability of vaccine-induced protection beyond 5 years is unknown. An antigen challenge of quadrivalent HPV-6/11/16/18 vaccine was shown to stimulate an anamnestic response, the hallmark of a vaccine that offers long-lasting protection.[31] The planned 15-year follow-up of vaccinated subjects in northern Europe[32] should provide essential information on the durability of protection.

Implementation of public health measures is challenging, owing to difficulties in maintaining compliance and to the heterogeneity of target populations. In this study, subjects were encouraged to comply with the three-dose vaccination regimen of administration at day 1, month 2, and month 6; however, subjects were not eliminated from the study if they deviated from this regimen. Analysis of the unrestricted susceptible population showed a high efficacy (95%), indicating that there is flexibility around the suggested regimen. Of note, it is likely that protective antibodies developed in some vaccinated women before they had received three doses, since infection with HPV-16 or HPV-18 before or within 1 month after receiving all three injections added 17 of 20 new cases in the placebo group and only 2 new cases in the vaccine group. On the other hand, there was no clear evidence that vaccination altered the course of HPV-16 or HPV-18 infection that was present before administration of the first vaccine dose.

It is likely that the population of women with prevalent HPV-16 or HPV-18 infection at trial entry was enriched for high-grade disease because high-grade lesions are more likely to persist than are low-grade lesions and because most HPV infections are transient.[33] Speculation that elimination of HPV-16 and HPV-18 will open a niche for other high-risk viruses is not supported by the literature. Young women are often infected with multiple high-risk HPV types, and the risk of new infection is greater for women who are infected with one or more HPV types than for uninfected women.[34-37] In addition, levels of viral DNA in clinical specimens may be higher for one HPV type when there is coinfection with another type.[38]

The quadrivalent vaccine is prophylactic, not therapeutic. Even in the setting of high vaccine coverage, routine screening for cervical cancer will be necessary to detect and treat disease caused by HPV-16 or HPV-18 infections acquired before vaccination and by other carcinogenic HPV types.

Prophylactic HPV vaccines must be safe and efficacious in diverse settings because women throughout the world are at risk for cervical cancer. In this trial and in a previous trial,[10] no safety concerns among nonpregnant women were identified. Additional data on vaccination during pregnancy are needed. Since women with human immunodeficiency virus infection or other immunosuppressive conditions were not enrolled in our study, future trials will be needed to evaluate safety and efficacy in these populations.

These data demonstrate that a quadrivalent HPV-6/11/16/18 vaccine was highly effective in preventing high-grade cervical lesions associated with HPV-16 and HPV-18. Widespread immunization of female children and adolescents may result in a substantial decrease in HPV-16–related and HPV-18–related cervical disease, including cervical cancer.

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APPENDIX

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