A Pooled Analysis of Continued Prophylactic Efficacy of Quadrivalent Human Papillomavirus (Types 6/11/16/18) Vaccine against High-grade Cervical and External Genital Lesions

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Abstract Quadrivalent human papillomavirus (HPV) vaccine has been shown to provide protection from HPV 6/11/16/18–related cervical, vaginal, and vulvar disease through 3 years. We provide an update on the efficacy of the quadrivalent HPV vaccine against high-grade cervical, vaginal, and vulvar lesions based on end-of-study data from three clinical trials. Additionally, we stratify vaccine efficacy by several baseline characteristics, including age, smoking status, and Papanicolaou (Pap) test results. A total of 18,174 females ages 16 to 26 years were randomized and allocated into one of three clinical trials (protocols 007, 013, and 015). Vaccine or placebo was given at baseline, month 2, and month 6. Pap testing was conducted at regular intervals. Cervical and anogenital swabs were collected for HPV DNA testing. Examination for the presence of vulvar and vaginal lesions was also done. Endpoints included high-grade cervical, vulvar, or vaginal lesions (CIN 2/3, VIN 2/3, or VaIN 2/3). Mean follow-up time was 42 months post dose 1. Vaccine efficacy against HPV 6/11/16/18–related high-grade cervical lesions in the per-protocol and intention-to-treat populations was 98.2% [95% confidence interval (95% CI), 93.3-99.8] and 51.5% (95% CI, 40.6-60.6), respectively. Vaccine efficacy against HPV 6/11/16/18–related high-grade vulvar and vaginal lesions in the per-protocol and intention-to-treat populations was 100.0% (95% CI, 82.6-100.0) and 79.0% (95% CI, 56.4-91.0), respectively. Efficacy in the intention-to-treat population tended to be lower in older women, women with more partners, and women with abnormal Pap test results. The efficacy of quadrivalent HPV vaccine against high-grade cervical and external anogenital neoplasia remains high through 42 months post vaccination.

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The worldwide burden of HPV infection (and diseases related to this infection) is substantial. Genital infection with human papillomavirus (HPV) is the most common sexually transmitted infection among women (1). A high proportion of sexually active persons will be infected with HPV during their lifetimes. A fundamental correlation between cervical HPV infection and cervical cancer is evident, as HPV can be found in more than 99% of cervical cancers (2). Whereas HPV-related cervical cancer is the second most common cancer among women worldwide (3), HPV infection can also lead to premalignant and malignant lesions of the vagina, vulva, and anus, as well as genital warts. The recognition of the viral etiology of cervical cancer and other genital diseases related to HPV has led to the development of prophylactic HPV vaccines directed against the most relevant disease-causing HPV types (4).

A small number of HPV types contribute to a large proportion of HPV-related genital disease. HPV types 16 and 18 are associated with ~70% of all invasive cervical cancers (5), and HPV types 6 and 11 are associated with 90% of all genital warts (6). A prophylactic quadrivalent HPV vaccine targeting HPV 6–, 11–, 16–, and 18–related cervical and external genital disease (as well as genital warts) has been developed and is now available in ~100 countries worldwide. Data show that this vaccine is highly effective in preventing HPV 6–, 11–, 16–, or 18–related cervical, vaginal, and vulvar intraepithelial neoplasias (CIN, VIN, VaIN) and cervical adenocarcinoma *in situ* (AIS), as well as genital warts in women naïve to the respective vaccine HPV types at enrollment (7–9).

Included in the application for vaccine licensure were efficacy summaries including data from three separate efficacy/immunogenicity trials (protocols 007, 013, and 015), which were similar in design and infrastructure and which mandated similar rigorous procedures for the collection of cervical and external genital lesion data. The data included in the initial application for vaccine licensure were based on the full 3 years of postvaccination follow-up in protocol 007 (9) and ~2 years of postvaccination follow-up in protocols 013 and 015. Subsequent updates on the efficacy of the vaccine through 3 years in protocols 013 and 015 have been published (7, 8). An update on the efficacy of the quadrivalent vaccine in protocol 007 through 5 years has also been published (10). Here, we provide an update on the efficacy of the quadrivalent HPV vaccine against high-grade cervical, vaginal, and vulvar lesions based on pooled final follow-up data from protocols 007, 013, and 015; we have also examined whether the impact of the vaccine changes according to different variables, such as age at enrollment, number of sexual partners, Papanicolaou (Pap) abnormality, smoking, and use of hormonal contraceptives.

Materials and Methods

Study design

The present analysis is representative of the combined final end-of-study data from three studies (007, 013, and 015). Protocol 007 (NCT n/a) was an international, randomized, double-blind, placebo-controlled phase IIb study designed to investigate the prophylactic efficacy of the quadrivalent HPV (types 6/11/16/18) L1 virus-like particle (VLP) vaccine (GARDASIL, Merck and Co., Inc.) on persistent infection or cervical/external genital disease associated with HPV 6, 11, 16, or 18, compared with placebo (9, 10). Protocols 013 (NCT00092521) and 015 (NCT00092534; termed FUTURE I and FUTURE II, respectively) were phase III, international, randomized, double-blind, placebo-controlled studies designed to investigate the prophylactic efficacy of the quadrivalent HPV (types 6/11/16/18) L1 VLP vaccine on HPV 6/11/16/18–related CIN, AIS, or cervical cancer (protocol 013 co-primary endpoint; ref. 7); HPV 6/11/16/18–related condyloma acuminata, VIN, VaIN, vulvar cancer, or vaginal cancer (protocol 013 co-primary endpoint; ref. 7); and HPV 16/18–related CIN 2/3, AIS, or cervical cancer (protocol 015 primary endpoint; ref. 8).

Female adolescents and young adults ages 16 to 26 years were enrolled into one of three clinical trials [protocols 007 (n = 1,106), 013 (n = 5,759), and 015 (n = 12,167)]. Participants enrolled were to be nonpregnant with no prior abnormal Pap smears. All subjects 18 years or older were to have a lifetime history of four or fewer lifetime sex partners. However, a proportion of 16- to 17-year-old Finnish girls had more than four lifetime sexual partners. Subjects with prior confirmed HPV disease were excluded from enrolling; however, those with prior or current subclinical HPV infection (through serology and PCR testing, respectively) were not excluded. Enrolled subjects with clinical evidence of external anogenital HPV disease at day 1 were discontinued from the study before randomization. All participants or parents/legal guardians signed informed consents following review of the protocol procedures. Participants received i.m. injections of quadrivalent HPV vaccine or visually indistinguishable placebo at enrollment (day 1), month 2, and month 6. The studies were conducted in conformance with applicable national or local requirements regarding ethical committee review, informed consent, and the protection of the rights and welfare of human subjects participating in biomedical research. A detailed description of the methodologies for each protocol included in the present analyses have been published previously (7, 8, 10).

Study vaccine

The quadrivalent vaccine consisted of a mixture of four recombinant HPV type–specific VLPs composed of the L1 major capsid proteins of HPV types 6, 11, 16, and 18 synthesized in *Saccharomyces cerevisiae* (11–13). The vaccine is composed of 20 μg of HPV 6 VLP, 40 μg of HPV 11 VLP, 40 μg of HPV 16 VLP, and 20 μg of HPV 18 VLP, in a total carrier volume of 0.5 mL. The four VLP types were purified and adsorbed onto amorphous aluminum hydroxyphosphate sulfate adjuvant. The placebo contained the same adjuvant and was visually indistinguishable from the vaccine.

Clinical follow-up and case definition

The mean follow-up time in the present analysis was 42 months post dose 1. Cervical and anogenital swabs were collected for HPV DNA testing at regular intervals. An examination for the presence of vulvar and vaginal lesions was also done. ThinPrep (Cytyc) cytology specimens for Pap testing were collected at enrollment (day 1), month 7, and at 6- to 12-month intervals thereafter. Cytology specimens were classified using the Bethesda System 2001 (14). Procedures for algorithm-based cytology, colposcopy, and biopsy referral have been described previously (7, 8). Biopsy material was first read for clinical management by pathologists at a central laboratory (Diagnostic Cytology Laboratories) and then read for endpoint determination by a blinded panel of four expert pathologists.

To be considered a case of HPV 6/11/16/18–related disease, subjects must have had a pathology panel consensus diagnosis of CIN 2 or worse, VIN 2/3 or worse, or VaIN 2/3 or worse with HPV 6, 11, 16, and/or 18 DNA detected (through multiplex PCR) in an adjacent section from the same tissue block.

Laboratory testing

Blood samples were obtained at enrollment (day 1) for anti-HPV serology testing for HPV types 6, 11, 16, and 18 using competitive

immunoassays (developed by Merck Research Laboratories, using technology from the Luminex Corporation; ref. 15). Dilution-corrected serostatus cutoffs were 20 mMU/mL for HPV 6, 16 mMU/mL for HPV 11, 20 mMU/mL for HPV 16, and 24 mMU/mL for HPV 18.

Ascertainment of HPV infection involved HPV PCR analysis done on genital swabs obtained at regular intervals. For each subject, genital swab specimens were tested for the presence of HPV 6, 11, 16, and 18 DNA. Swabs, biopsy fragments, and tissue thin sections cut adjacent to sections used for histopathology were used for HPV DNA detection using HPV 6–, 11–, 16–, or 18–specific L1, E6, and E7 primers in HPV multiplex real-time PCR assays (9).

Study differences

Although protocols 007, 013, and 015 are similar in many ways, there are differences that should be acknowledged. In protocol 013, an additional study visit at month 3 could potentially have caused more subjects to be excluded from the per-protocol analysis due to PCR positivity for the vaccine HPV types than when compared with protocols 007 and 015. However, this did not seem to be the case. In protocol 007, colposcopies and biopsies were done based on voluntary Pap triage guidelines, whereas protocols 013 and 015 had mandatory Pap triage guidelines. Protocol 015 required Pap screening every 12 months, whereas protocols 007 and 013 required Pap screening every 6 months.

Statistical analysis

The primary analysis of efficacy was conducted in type-specific per-protocol populations that consisted of subjects who were PCR negative and seronegative to HPV 6, HPV 11, HPV 16, or HPV 18 at enrollment; remained PCR negative to the same vaccine HPV type (s), to which they were naïve at enrollment, through 1 month post dose 3; received three doses of vaccine or placebo within 1 year; and did not violate the protocol. Case counting for this population started at month 7. For example, to be eligible for the per-protocol population for HPV 16–related endpoints, a subject had to be both HPV 16 seronegative and HPV 16 DNA negative by PCR at enrollment, and remain HPV 16 DNA negative through month 7.

Supportive analyses of an intention-to-treat population were also conducted. To be eligible for this population, subjects must have received ≥1 dose of vaccine or placebo and have returned for follow-up after day 1. Case counting for this population started after day 1. Subjects in this population could have been infected with vaccine HPV types at enrollment or before month 7 and could have also had disease related to vaccine HPV types at day 1 or before month 7.

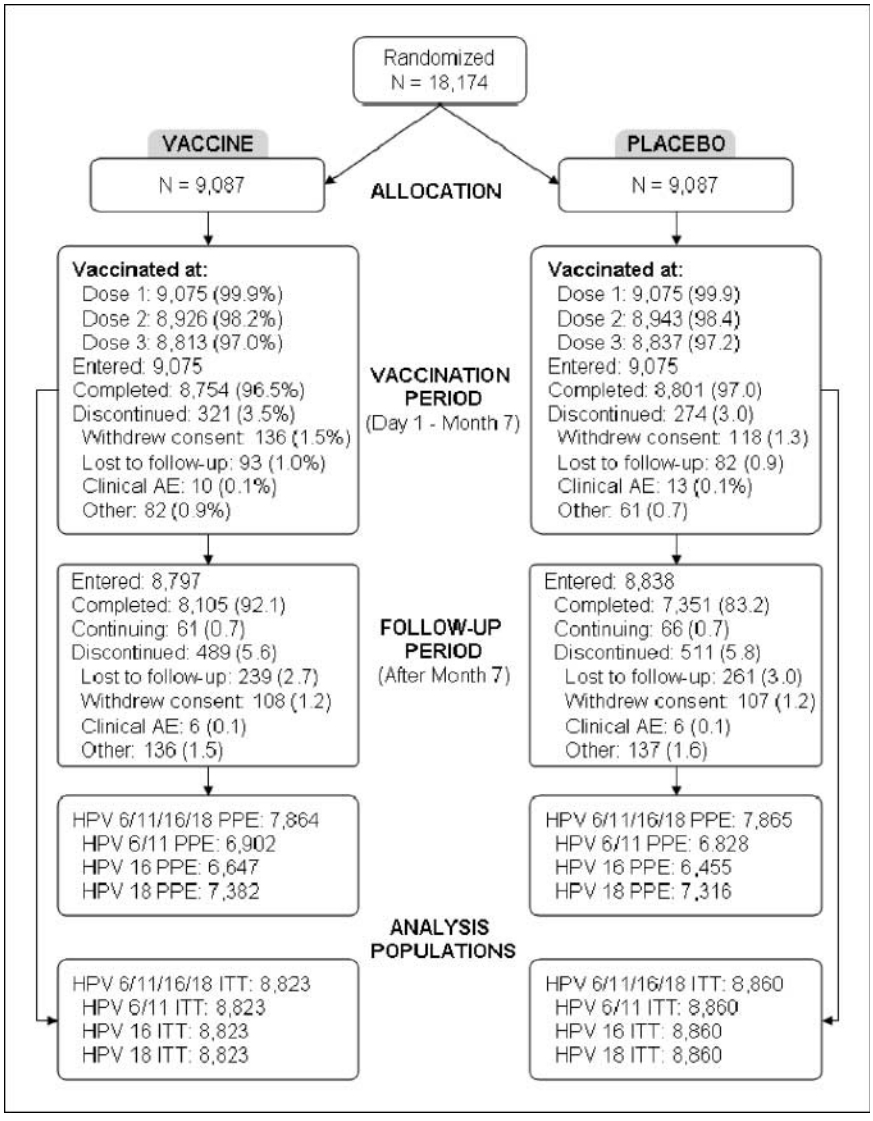

Fig. 1. Subject accounting in protocols 007, 013, and 015. For the follow-up period, there were an additional 142 (1.6%) subjects that received quadrivalent HPV vaccine and 910 (10.3%) subjects that received placebo that completed the study and entered an extended phase where they get quadrivalent HPV vaccine. In addition, a status of "continuing" indicates completion of study visits associated with end of study; however, these subjects had unknown pregnancy outcomes at the time of this report. *PPE*, per-protocol efficacy population; *AE*, adverse experience.

Table 1. Baseline characteristics of participants in the study population (protocols 007, 013, and 015)

Baseline characteristic	Quadrivalent HPV vaccine (*N* = 9,087)	Placebo (*N* = 9,087)
Age (y)		
Mean ± SD	20.0 ± 2.0	20.0 ± 2.0
Median (range)	20 (15-26)	20 (13-26)
Race, *m/n* (%)		
Asian	309/9,087 (3.4%)	311/9,087 (3.4%)
Black	332/9,087 (3.7%)	412/9,087 (4.5%)
Hispanic American	1,136/9,087 (12.5%)	1,143/9,087 (12.6%)
Native American	13/9,087 (0.1%)	12/9,087 (0.1%)
White	6,400/9,087 (70.4%)	6,322/9,087 (69.6%)
Other	897/9,087 (9.9%)	887/9,087 (9.8%)
Lifetime number of sex partners at enrollment, *m/n* (%)		
0 (Virgin)	517/9,087 (5.7%)	561/9,087 (6.2%)
1	2,929/9,087 (32.2%)	2,938/9,087 (32.3%)
2	2,210/9,087 (24.3%)	2,224/9,087 (24.5%)
3	1,798/9,087 (19.8%)	1,754/9,087 (19.3%)
4	1,519/9,087 (16.7%)	1,477/9,087 (16.3%)
>4	111/9,087 (1.2%)	128/9,087 (1.4%)
Unknown	3/9,087 (0.0%)	5/9,087 (0.1%)
New sex partners in 6 mo before study start,* *m/n* (%)		
0	5,990/8,570 (69.9%)	5,881/8,526 (69.0%)
1	2,196/8,570 (25.6%)	2,231/8,526 (26.2%)
2	307/8,570 (3.6%)	317/8,526 (3.7%)
3	50/8,570 (0.6%)	64/8,526 (0.8%)
4	12/8,570 (0.1%)	16/8,526 (0.2%)
>4	11/8,570 (0.1%)	13/8,526 (0.2%)
Unknown	4/8,570 (0.0%)	4/8,526 (0.0%)
Prevalence of sexually transmitted disease, *m/n* (%)		
Chlamydia	379/8,939 (4.2%)	367/8,915 (4.1%)
Gonorrhea	35/6,764 (0.5%)	23/6,742 (0.3%)
Pregnancy history, *m/n* (%)		
With history of pregnancy	2,057/9,087 (22.6%)	2,041/9,087 (22.5%)
Day 1 Pap status, *m/n* (%)		
ASC-US or worse before study or on day 1	1,003/8,691 (11.5%)	986/8,658 (11.4%)
ASC-US or worse with positive high-risk HPV probe	47/8,691 (0.5%)	66/8,658 (0.8%)
HPV positivity at day 1		
PCR positive, *m/n* (%)		
Positive to HPV 6/11/16/18	1,329/8,983 (14.8%)	1,317/8,997 (14.6%)
Positive to HPV 6	372/8,962 (4.2%)	348/8,977 (3.9%)
Positive to HPV 11	66/8,978 (0.7%)	55/8,992 (0.6%)
Positive to HPV 16	781/8,932 (8.7%)	783/8,967 (8.7%)
Positive to HPV 18	317/8,969 (3.5%)	323/8,986 (3.6%)
Seropositive, *m/n* (%)		
Positive to HPV 6/11/16/18	1,792/9,067 (19.8%)	1,788/9,066 (19.7%)
Positive to HPV 6	738/9,067 (8.1%)	745/9,066 (8.2%)
Positive to HPV 11	189/9,066 (2.1%)	187/9,066 (2.1%)
Positive to HPV 16	1,001/9,067 (11.0%)	1,037/9,066 (11.4%)
Positive to HPV 18	332/9,066 (3.7%)	338/9,066 (3.7%)
PCR positive and/or seropositive		
Positive to HPV 6/11/16/18	2,434/9,074 (26.8%)	2,418/9,074 (26.6%)
Positive to HPV 6	962/9,074 (10.6%)	962/9,074 (10.6%)
Positive to HPV 11	231/9,074 (2.5%)	223/9,074 (2.5%)
Positive to HPV 16	1,433/9,074 (15.8%)	1,445/9,074 (15.9%)
Positive to HPV 18	574/9,074 (6.3%)	572/9,074 (6.3%)

NOTE: *N* is the number of subjects randomized to the respective vaccination group; *n* is the number of subjects with nonmissing day 1 result with respect to the relevant method assessment of the given characteristic; and *m* is the number of subjects who were positive at day 1 with respect to the relevant method assessment of the given characteristic.

Abbreviation: ASC-US, atypical squamous cells of undetermined significance.

*Among nonvirgins.

Vaccine efficacy [defined as (1 − relative risk) × 100%] and the corresponding 95% confidence intervals (95% CI) were estimated using an exact procedure that accounted for the amount of follow-up (i.e., person-time at risk) in the vaccine and placebo groups. Subjects were pooled across the studies by vaccination group (vaccine or placebo) for analysis.

Results

A total of 18,174 participants were randomized and allocated to quadrivalent HPV vaccine or placebo (Fig. 1), with 96.5% and 97.0% of subjects allocated to vaccine or placebo completing the three dose series, respectively. At the time of this report, 92.1% of subjects receiving vaccine and 83.2% of subjects receiving placebo had completed follow-up (of those who entered follow-up). Of the subjects who entered the vaccination phase of protocols 007, 013, and 015, 86.7% were included in the per-protocol analysis for the composite endpoint of HPV 6/11/16/18–related disease.

Baseline characteristics of all enrolled subjects can be seen in Table 1. In general, the vaccine and placebo groups were very similar with respect to age, race, numbers of sexual partners, prevalence of sexually transmitted infection, and other factors measured.

Efficacy against CIN 2/3

Vaccine efficacy against HPV 6/11/16/18–related high-grade cervical lesions in the per-protocol population was 98.2% (95% CI, 93.3-99.8; Table 2). There were two cases of CIN 2 or worse related to vaccine HPV types in subjects receiving vaccine in the per-protocol population. Both of these cases were HPV 16–related CIN 3. One case was PCR positive for HPV 51 and negative for other tested types at enrollment. A colposcopy specimen taken at month 49 was positive for HPV 16 only. Definitive therapy was done at month 52; this revealed a negative pathology panel diagnosis, which was positive for HPV 56 and negative for HPV 16 and all other tested types. The other case was PCR positive for HPV 52 and negative for vaccine HPV types at enrollment. A colposcopy specimen taken at month 32.5 was positive for both HPV 52 and HPV 16. All specimens collected at definitive therapy were HPV 52 positive and negative for all other tested types (including HPV 16 negative). As a consequence of these two cases, the efficacy of the vaccine against HPV 16–related high-grade cervical lesions in this population was 97.6% (95% CI, 91.1-99.7). Efficacy against high-grade cervical lesions related to each of the other vaccine HPV types in the per-protocol population was 100.0%. In the intention-to-treat population, the reduction in HPV 6/11/16/18–related high-grade cervical lesions was 51.5% (95% CI, 40.6-60.6). The majority of cases in both the vaccine and placebo subgroups of the intention-to-treat population were related to HPV 16. The incidence rate of HPV 6/11/16/18–related CIN 2 or worse over time among vaccine recipients and placebo recipients in the intention-to-treat population can be seen in Fig. 2A. The percentage of women with HPV 6/11/16/18–related CIN 2 or worse was similar in the vaccine and the placebo group in the 1st year after vaccination, presumably due to prevalent

Table 2. Vaccine efficacy against HPV 6–, 11–, 16–, and 18–related high-grade cervical lesions (CIN 2 or worse) through the end of the study (protocols 007, 013, and 015) by severity and HPV type

	Quadrivalent HPV vaccine (*N* = 9,075)			Placebo (*N* = 9,075)			Observed efficacy, % (95% CI)
	n	Cases	Rate	*n*	Cases	Rate	
Per-protocol							
By lesion severity							
CIN 2 or worse	7,864	2	0.0	7,865	110	0.5	98.2 (93.3-99.8)
CIN 2	7,864	0	0.0	7,865	71	0.3	100.0 (94.7-100.0)
CIN 3 or worse	7,864	2	0.0	7,865	66	0.3	97.0 (88.7-99.6)
CIN 3	7,864	2	0.0	7,865	63	0.3	96.8 (88.1-99.6)
AIS	7,864	0	0.0	7,865	7	0.0	100.0 (30.9-100.0)
By HPV type							
HPV 6	6,902	0	0.0	6,828	12	0.1	100.0 (64.5-100.0)
HPV 11	6,902	0	0.0	6,828	4	0.0	100.0 (<0-100.0)
HPV 16	6,647	2	0.0	6,455	81	0.4	97.6 (91.1-99.7)
HPV 18	7,382	0	0.0	7,316	29	0.1	100.0 (86.6-100.0)
Intention-to-treat							
By lesion severity							
CIN 2 or worse	8,823	142	0.5	8,860	293	1.0	51.5 (40.6-60.6)
CIN 2	8,823	81	0.3	8,860	183	0.6	55.7 (42.2-66.4)
CIN 3 or worse	8,823	104	0.3	8,860	195	0.6	46.6 (32.0-58.3)
CIN 3	8,823	102	0.3	8,860	186	0.6	45.1 (29.8-57.3)
AIS	8,823	6	0.0	8,860	15	0.0	60.0 (<0-87.3)
By HPV type							
HPV 6	8,823	2	0.0	8,860	23	0.1	91.3 (64.8-99.0)
HPV 11	8,823	0	0.0	8,860	6	0.0	100.0 (15.0-100.0)
HPV 16	8,823	132	0.4	8,860	243	0.8	45.7 (32.6-56.4)
HPV 18	8,823	9	0.0	8,860	60	0.2	85.0 (69.6-93.5)

NOTE: Subjects are counted once in each applicable endpoint category. A subject may appear in more than one category. *N* is the number of subjects randomized to the respective vaccination group who received at least one injection; *n* is the number of subjects evaluable (i.e., number of subjects in the given population who also have at least one follow-up visit). "Rate" is the rate per 100 person-years at risk.
Abbreviation: CIN, cervical intraepithelial neoplasia.

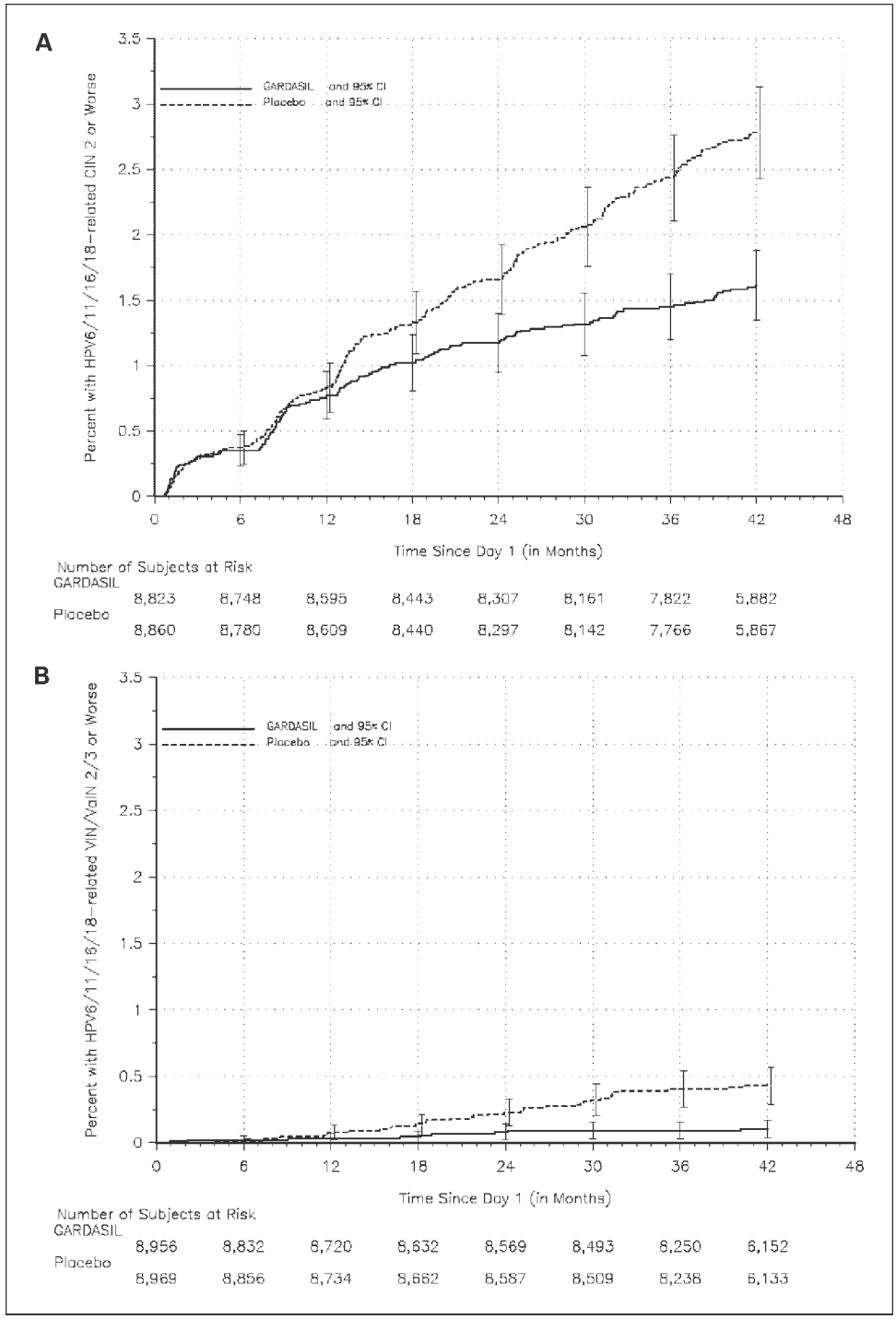

Fig. 2. Time-to-event curves for (*A*) CIN 2 or worse related to HPV 6/11/16/18 and (*B*) VIN 2/3 or VaIN 2/3 related to HPV 6/11/16/18 in the intention-to-treat populations of protocols 007, 013, and 015.

infection or disease. However, subsequently, a statistically significant reduction in HPV 6/11/16/18–related CIN 2 or worse became apparent in the vaccination group compared with the placebo group.

Stratification of vaccine efficacy against HPV 6/11/16/18–related high-grade cervical lesions by various baseline subject characteristics is presented in Table 3. In the per-protocol population, the efficacy was very similar for all stratifications. In the intention-to-treat population, efficacy tended to decrease with age, in women with increasing lifetime number of lifetime sex partners, in women with a history of smoking or abnormal Pap test results, and in those using hormonal contraceptives at day 1. When examining the prevalence of HPV 6/11/16/18 positivity by different levels of these variables, we found that the proportion of women being positive for one or more of these vaccine HPV types at baseline increased both with smoking [from 12.6% (95% CI, 12.0-13.2) in never smokers to 19.4% (95% CI,

Table 3. Analysis of efficacy against HPV 6/11/16/18–related high-grade cervical lesions (CIN 2 or worse) by categories of baseline covariates

	Per-protocol population					Intention-to-treat population				
	Quadrivalent HPV vaccine		Placebo		Efficacy, % (95% CI)	Quadrivalent HPV vaccine		Placebo		Reduction, % (95% CI)
	Cases/*n*	Rate	Cases/*n*	Rate		Cases/*n*	Rate	Cases/*n*	Rate	
No. of sexual partners										
0	0/464	0.0	8/508	0.5	100.0 (35.9-100.0)	1/500	0.1	8/540	0.4	86.5 (<0.0-99.7)
1-2	2/4,502	0.0	59/4,509	0.4	96.6 (87.3-99.6)	58/4,997	0.3	127/5,040	0.7	54.4 (37.3-65.1)
≥3	0/2,898	0.0	43/2,845	0.5	100.0 (91.2-100.0)	83/3,321	0.7	158/3,274	1.4	48.1 (31.8-60.7)
Covariate by efficacy interaction					$P = 0.571$					$P = 0.395$
Age at start of vaccination (y)										
≤17	0/1,039	0.0	22/1,057	0.7	100.0 (81.6-100.0)	11/1,126	0.3	36/1,150	0.9	69.0 (37.8-85.8)
18-20	0/3,258	0.0	56/3,313	0.6	100.0 (93.1-100.0)	57/3,690	0.5	151/3,749	1.2	61.9 (47.9-72.4)
≥21	0/3,567	0.0	32/3,495	0.3	93.9 (76.1-99.3)	74/4,007	0.5	106/3,961	0.8	31.1 (6.4-49.5)
Covariate by efficacy interaction					$P = 0.131$					$P = 0.009$
History of or day 1 abnormal Pap										
Normal	2/6,737	0.0	91/6,739	0.5	97.8 (91.9-99.7)	58/7,462	0.2	188/7,488	0.7	69.2 (58.4-77.4)
Abnormal	0/815	0.0	15/784	0.7	100.0 (73.9-100.0)	78/991	2.6	92/965	3.2	18.7 (<0.0-40.7)
Covariate by efficacy interaction					$P = 1.00$					$P < 0.001$
Smoking history										
Without smoking history	0/2,600	0.0	42/2,700	0.5	100.0 (90.5-100.0)	72/5,865	0.4	164/5,756	1.2	57.0 (43.0-67.9)
With smoking history	2/5,263	0.0	68/5,163	0.5	97.1 (89.2-99.7)	70/2,952	0.7	129/3,099	0.8	43.4 (23.7-58.3)
Covariate by efficacy interaction					$P = 0.534$					$P = 0.183$
Hormonal contraception use										
No hormonal contraception at day 1	1/4,661	0.0	69/4,652	0.5	98.6 (91.7-100.0)	41/3,575	0.6	107/3,625	1.0	61.3 (44.1-73.7)
Hormonal contraception at day 1	1/3,193	0.0	41/3,196	0.4	97.6 (85.6-99.9)	101/5,230	0.3	186/5,211	0.9	46.1 (31.0-58.1)
Covariate by efficacy interaction					$P = 1.000$					$P = 0.160$

NOTE: *n* is the number of subjects evaluable (i.e., number of subjects in the given population who also have at least one follow-up visit). "Rate" is the rate per 100 person-years at risk.

18.3-20.5) in current smokers] and with number of lifetime sex partners [from 1.6% (95% CI, 0.85-2.4) in virgins, to 7.7% (95% CI, 7.0-8.4) in women with one lifetime sex partner, to 26.4% (95% CI, 24.9-28.0) in women reporting four lifetime sex partners at baseline].

Efficacy against VIN 2/3 and VAIN 2/3

Vaccine efficacy against HPV 6/11/16/18–related high-grade vulvar and vaginal lesions in the per-protocol population was 100.0% (95% CI, 82.6-100.0; Table 4). No cases were seen in vaccinated subjects, whereas 23 cases of VIN or VaIN were diagnosed in those subjects receiving placebo. In the intention-to-treat population, the efficacy against high-grade vulvar and vaginal lesions was 79.0% (95% CI, 56.4-91.0). The majority of VIN or VaIN cases seen in both the per-protocol and intention-to-treat populations were related to HPV 16. The incidence rate of HPV 6/11/16/18–related high-grade vulvar and vaginal lesions over time among vaccine recipients and placebo recipients in the intention-to-treat population can be seen in Fig. 2B.

Table 5 presents vaccine efficacy against HPV 6/11/16/18–related high-grade vulvar and vaginal lesions stratified by baseline covariates. The pattern was similar to results with high-grade cervical lesions; however, the differences observed between the results for the per-protocol population and the intention-to treat population were less pronounced.

Additional analyses of efficacy against high-grade cervical, vulvar, or vaginal disease in the per-protocol and intention-to-treat populations stratified by further covariates can be seen in Supplementary Material. These covariates include sexual partners in the 6 months before vaccination and baseline HPV status (both DNA and serology).

Discussion

These findings show that quadrivalent HPV (types 6/11/16/18) L1 VLP vaccine provides sustained high prophylactic efficacy against high-grade cervical, vaginal, and vulvar intraepithelial neoplasia through a mean follow-up of 42 months. We analyzed data in both a per-protocol (HPV 6/11/16/18 naïve) population and an intention-to-treat (both HPV nonnaïve and HPV naïve) population. Efficacy was greatest in the per-protocol population; this result is not unexpected and is discussed below. It is important to note that although the clinical trials included a broad representation of women, 16- to 26-year-old women with high numbers of sex partners or with poor access to health care were underrepresented. Thus, the findings of this analysis cannot be immediately extrapolated to all women.

Stratifying our efficacy results by different baseline characteristics allowed us to determine if the vaccine provided similar efficacy in different subsets of women. In the intention-to-treat population, we found a tendency toward a decreased disease reduction with increasing lifetime number of sex partners and smoking. As both of these baseline covariates are most likely serving as surrogates for HPV exposure at baseline, these data are consistent with previous findings of lower efficacy with HPV positivity before vaccination. In support of this hypothesis, we found indeed that the proportion of women being positive for one or more of the vaccine HPV types at baseline increased both with smoking and with number of lifetime sex partners. Whereas vaccine efficacy tended to be lower in women with more than one lifetime sex partner, a similar amount of disease was prevented in the sexually active women when compared with the women with 0 lifetime sex partners based on absolute risk (placebo rate – vaccine rate; Table 3, intention-to-treat population).

Efficacy stratified by baseline Pap status showed a reduced efficacy in women with baseline abnormal Pap results (HSIL, ASC-H, LSIL, AAGC, and ASC-US) in the intention-to-treat population. It is not surprising that the impact of vaccination was lower among subjects with a Pap test abnormality at vaccination onset because many of these women already had HPV disease at vaccination onset or experienced such disease as a consequence of infections that were present already, and the HPV vaccine will not alter the course of infection or disease that is present already at the onset of vaccination. However, even among the 159 subjects with atypical squamous cells, we cannot rule out high-grade squamous intraepithelial lesions (ASC-H) or high-grade squamous intraepithelial lesions; only a small proportion of subjects (8 of 159 women; 5.0%) were positive for three of the four vaccine HPV types. Only

Table 4. Vaccine efficacy against HPV 6–, 11–, 16–, and 18–related high-grade vulvar and vaginal lesions through the end of the study (protocols 007, 013, and 015) by severity and HPV type

	Quadrivalent HPV vaccine (*N* = 9,075)			Placebo (*N* = 9,075)			Observed efficacy, % (95% CI)
	n	Cases	Rate	*n*	Cases	Rate	
Per-protocol							
By lesion severity							
VIN 2/3, VaIN 2/3 or worse	7,900	0	0.0	7,902	23	0.1	100.0 (82.6-100.0)
VIN 2/3	7,900	0	0.0	7,902	13	0.1	100.0 (67.2-100.0)
VaIN 2/3	7,900	0	0.0	7,902	10	0.0	100.0 (55.4-100.0)
By HPV type							
HPV 6	6,932	0	0.0	6,856	6	0.0	100.0 (15.8-100.0)
HPV 11	6,932	0	0.0	6,856	1	0.0	100.0 (<0-100.0)
HPV 16	6,654	0	0.0	6,467	17	0.1	100.0 (76.5-100.0)
HPV 18	7,414	0	0.0	7,343	2	0.0	100.0 (<0-100.0)
Intention-to-treat							
By lesion severity							
VIN2/3, VaIN2/3 or worse	8,956	9	0.0	8,969	43	0.1	79.0 (56.4-91.0)
VIN2/3	8,956	8	0.0	8,969	30	0.1	73.3 (40.3-89.4)
VaIN2/3	8,956	2	0.0	8,969	14	0.0	85.7 (37.6-98.4)
By HPV type							
HPV 6	8,956	1	0.0	8,969	9	0.0	88.9 (19.5-99.7)
HPV 11	8,956	0	0.0	8,969	2	0.0	100.0 (<0-100.0)
HPV 16	8,956	8	0.0	8,969	36	0.1	77.7 (51.2-91.1)
HPV 18	8,956	1	0.0	8,969	3	0.0	66.5 (<0-99.4)

NOTE: Subjects are counted once in each applicable endpoint category. A subject may appear in more than one category. *N* is the number of subjects randomized to the respective vaccination group who received at least one injection; *n* is the number of subjects evaluable (i.e., number of subjects in the given population who also have at least one follow-up visit). "Rate" is the rate per 100 person-years at risk. Abbreviations: VIN, vulvar intraepithelial neoplasia; VaIN, vaginal intraepithelial neoplasia.

Table 5. Analysis of efficacy against HPV 6/11/16/18–related high-grade vulvar and vaginal lesions by categories of baseline covariates

	Per-protocol population					Intention-to-treat population				
	Quadrivalent HPV vaccine		Placebo		Efficacy, % (95% CI)	Quadrivalent HPV vaccine		Placebo		Reduction, % (95% CI)
	Cases/*n*	Rate	Cases/*n*	Rate		Cases/*n*	Rate	Cases/*n*	Rate	
No. of sexual partners										
0	0/464	0.0	1/509	0.1	100.0 (<0.0-100.0)	0/512	0.0	1/555	0.1	100.0 (<0.0-100.0)
1-2	0/4,514	0.0	14/4,526	0.1	100.0 (69.8-100.0)	5/5,067	0.0	25/5,089	0.1	79.9 (46.6-94.0)
≥3	0/2,922	0.0	8/2,864	0.1	100.0 (42.6-100.0)	4/3,372	0.0	17/3,319	0.1	76.8 (28.9-94.3)
Covariate by efficacy interaction					$P = 1.000$					$P = 1.000$
Age at start of vaccination (y)										
≤17	0/1,044	0.0	5/1,061	0.2	100.0 (<0.0-100.0)	1/1,138	0.0	10/1,151	0.2	89.9 (28.7-99.8)
18-20	0/3,275	0.0	12/3,332	0.1	100.0 (63.4-100.0)	3/3,758	0.0	22/3,816	0.2	86.2 (54.0-97.4)
≥21	0/3,581	0.0	6/3,509	0.1	100.0 (16.7-100.0)	5/4,060	0.0	11/4,002	0.1	55.1 (<0.0-87.8)
Covariate by efficacy interaction					$P = 1.000$					$P = 0.311$
History of or day 1 abnormal Pap										
Normal	0/6,734	0.0	19/6,741	0.1	100.0 (78.6-100.0)	5/7,589	0.0	34/7,585	0.1	85.3 (62.2-95.5)
Abnormal	0/852	0.0	2/818	0.1	100.0 (<0.0-100.0)	4/996	0.1	5/976	0.1	22.1 (<0.0-84.6)
Covariate by efficacy interaction					$P = 1.000$					$P = 0.053$
Smoking history										
Without smoking history	0/2,617	0.0	8/2,714	0.1	100.0 (39.4-100.0)	7/5,946	0.0	26/5,824	0.2	73.6 (37.5-90.3)
With smoking history	0/5,282	0.0	15/5,186	0.1	100.0 (72.6-100.0)	2/3,004	0.0	17/3,140	0.1	87.7 (48.3-98.6)
Covariate by efficacy interaction					$P = 1.000$					$P = 0.465$
Hormonal contraception use										
No hormonal contraception at day 1	0/4,688	0.0	12/4,673	0.1	100.0 (64.2-100.0)	5/3,649	0.0	23/3,681	0.1	87.9 (40.7-93.4)
Hormonal contraception at day 1	0/3,202	0.0	11/3,212	0.1	100.0 (59.9-100.0)	4/5,289	0.0	20/5,262	0.2	80.1 (40.7-95.1)
Covariate by efficacy interaction					$P = 1.000$					$P = 1.000$

NOTE: Subjects are counted once in each applicable endpoint category. A subject may appear in more than one category. *n* is the number of subjects evaluable (i.e., number of subjects in the given population who also have at least one follow-up visit). "Rate" is the rate per 100 person-years at risk.

one of these women was positive to all four types. Thus, most 16- to 26-year-old women will benefit from protection against all four vaccine HPV types, and a large majority will benefit from protection against three of four vaccine HPV types.

The effect of hormonal contraceptive usage on HPV infection and HPV-related disease susceptibility has been a topic of much research (16–19). Data to date are contradictory, however, on whether the usage of such contraceptives increases or decreases HPV-related infection or disease risk. The current analysis shows that there are no statistically significant differences in HPV vaccine efficacy between those women who are or are not taking hormonal contraceptives. In the per-protocol population, no variation in efficacy was observed for the different strata, including lifetime number of sex partners or smoking history.

Differences between vaccine efficacy in the per-protocol and intention-to-treat populations imply that the maximum impact from prophylactic HPV vaccination programs will be achieved in women susceptible to infection and disease related to vaccine HPV types (those not previously exposed), as has previously been suggested (20–22). The vaccine is prophylactic and is not expected to have a therapeutic effect on current HPV infection (HPV DNA positivity) or high-grade intraepithelial lesions/cancer. However, as shown in the time-to-event analysis in the intention-to-treat population (which comprises all combinations of HPV PCR–positive/negative, serologically positive/negative, and HPV-naïve women), a significantly lower incidence of HPV 16/18–related CIN2 or worse was seen over time in the vaccination group. These results confirm previous data showing that the vaccine

can confer cervical, vaginal, and vulvar cancer prophylaxis (20) and also show that vaccine-induced reduction in cervical, vaginal, and vulvar neoplasia is becoming more evident over time in the intention-to-treat population as prevalent infections resolve and placebo recipients, but not vaccine recipients, continue to have high rates of vaccine-type infections and lesions. In addition, the quadrivalent HPV vaccine may be beneficial in those subjects who are not naïve to all vaccine-related types, as it has been shown that subjects infected with ≥1 vaccine HPV type(s) are protected from disease with the remaining vaccine HPV types (23).

In conclusion, we have shown that the efficacy of quadrivalent HPV vaccine against high-grade cervical and external anogenital intraepithelial neoplasia remains high in the per-protocol population through 42 months post vaccination. Additionally, the benefit of vaccination in the intention-to-treat population became more apparent over time as vaccination prevented new infections with the vaccine HPV types resulting in a significantly lower proportion of women developing CIN 2 or worse and VIN 2/3 or VaIN 2/3 related to HPV 6/11/16/18 during follow-up among vaccine recipients compared with placebo recipients. Follow-up studies will be needed to determine the long-term efficacy of the vaccine, and those studies are currently under way. Stratification of vaccine efficacy by baseline characteristics in the per-protocol population showed consistent efficacy for all strata. Reduction in cervical disease in the intention-to-treat population tended to be lower in older women, women with more sexual partners, and in women with abnormal Pap results, likely due to increased baseline HPV virus exposure in these women.

Disclosure of Potential Conflicts of Interest

N. Muñoz has received lecture fees, advisory board fees, and consultancy fees from Merck and Sanofi Pasteur MSD. S.-E. Olsson has received lecture fees from Merck. M. Hernandez-Avila has received lecture fees and grant support from Merck. O.-E. Iversen has received lecture fees from Merck and GlaxoSmithKline. C.M. Wheeler has received funding through her institution to conduct HPV vaccine studies for GlaxoSmithKline and Roche Molecular Systems. K.A. Ault has received consultancy and advisory board fees. F.X. Bosch has received lecture fees from Merck and GlaxoSmithKline and has received funding through his institution to conduct HPV vaccine studies GlaxoSmithKline. J. Paavonen has received consultancy fees, advisory board fees, and lecture fees from Merck. J. Dillner has received consultancy fees, lecture fees, and research grants from Merck and Sanofi Pasteur MSD. S. Leodolter has received lecture fees from Merck and Sanofi Pasteur MSD. E. Joura has received lecture fees from Merck, Sanofi Pasteur MSD, and GlaxoSmithKline. S.K. Kjaer has received consultancy fees and has received funding through her institution to conduct HPV studies for Sanofi Pasteur MSD and Digene. S.M. Garland has received advisory board fees and grant support from Commonwealth Serum Laboratories and GlaxoSmithKline and lecture fees from Merck. D.G. Ferris has received consultancy fees and funding through his institution to conduct HPV vaccine studies for GlaxoSmithKline and lecture fees and consultancy fees from Merck. K. Sigurdsson has received consultancy fees from Merck. S. Majewski has received lecture fees and advisory board fees from Merck. G. Perez has received lecture fees and consultancy fees from Merck and Sanofi Pasteur MSD. D.R. Brown has received lecture fees, advisory board fees, and intellectual property fees. M. Steben has received lecture fees and grant support from Merck. Additionally, S.-E. Olsson, C.M. Wheeler, M. Hernandez-Avila, L.L. Villa, O.-E. Iversen, G.W.K. Tang, F.X. Bosch, J. Paavonen, J. Dillner, M. Lehtinen, E.H. Tay, S. Leodolter, E. Joura, S.K. Kjaer, G. Perez, D.G. Ferris, K. Sigurdsson, M. Steben, L.A. Koutsky, and D.R. Brown have received funding through their institutions to conduct HPV vaccine studies for Merck. F.J. Taddeo, C. Roberts, A. Tadesse, J. Bryan, R. Maansson, S. Vuocolo, S. Leodolter, T.M. Hesley, A. Saah, R.M. Haupt, and E. Barr are employees of Merck and potentially own stock and/or stock options in the company.

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