Safety and Persistent Immunogenicity of a Quadrivalent Human Papillomavirus Types 6, 11, 16, 18 L1 Virus-Like Particle Vaccine in Preadolescents and Adolescents

A Randomized Controlled Trial

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Objective: Administration of a quadrivalent HPV-6/11/16/18 vaccine to 16- to 26-year-old women was highly effective in preventing HPV-6/11/16/18-related cervical/vulvar/vaginal precancerous lesions and genital warts. As the risk of acquiring HPV significantly rises after sexual debut, HPV vaccines should have the greatest benefit in sexually naive adolescents. We evaluated the tolerability and immunogenicity of quadrivalent vaccine in males and females 9 to 15 years of age through 18 months postenrollment.

Methods: In this randomized, double-blind trial, 1781 sexually naive children were assigned (2:1) to quadrivalent HPV-6/11/16/18 vaccine or saline placebo administered at day 1 and months 2 and 6. Serum neutralizing anti-HPV-6/11/16/18 responses were summarized as geometric mean titers (GMTs) and seroconversion rates. Primary analyses were done per-protocol (subjects received 3 doses, had no major protocol violations and were HPV type-specific seronegative at day 1). Adverse experiences were collected by diary card.

Results: At month 7, seroconversion rates were ≥99.5% for the 4 vaccine-HPV-types. GMTs and seroconversion rates in boys were noninferior to those in girls ($P < 0.001$). At month 18, ≥91.5% of vaccine recipients were seropositive, regardless of gender. A higher proportion of vaccine recipients (75.3%) than placebo recipients (50.0%) reported one or more injection-site adverse experiences following any vaccination. Rates of fever were similar between vaccination groups. No serious vaccine-related adverse experiences were reported.

Conclusions: In 9- to 15-year-old adolescents, the quadrivalent vaccine was generally well tolerated and induced persistent anti-HPV serologic responses in the majority of subjects for at least 12 months following completion of a three-dose regimen. The vaccine durability supports universal HPV vaccination programs in adolescents to reduce the burden of clinical HPV disease, particularly cervical cancer and precancers.

Key Words: HPV, vaccine, immunogenicity, reactogenicity, pediatric, noninferiority

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Human papillomavirus (HPV) infection causes cervical cancer and genital warts.[1–6] HPV infection is common, with a lifetime risk exceeding 50% for sexually active males and females.[7] Studies have shown that the 5 years following sexual debut represent the period of highest risk for acquisition of HPV infection.[8–10] In most countries, the median/mean age of sexual debut is between 15 and 16 years of age.[11,12] Because HPV is the major cause of cervical cancer, the high prevalence of genital HPV infection is considered a serious worldwide health issue.[7]

The genital HPV family is composed of ~35 distinct types. These HPV types are divided into high risk (associated with the development of anogenital cancers) and low risk (associated with the development of dysplasia and anogenital warts, but rarely cancer). Four HPV types have been associated with the majority of HPV-related clinical disease. HPV types 16 and 18 cause approximately 70% of cervical cancers, HPV-6 and HPV-11 cause approximately 90% of genital warts (men and women) and types HPV-6/11/16/18 together cause a significant proportion of cervical intraepithelial neoplasia (CIN) leading to abnormal Papanicolaou (Pap) smears.[2,5,14–16]

The well-established link between HPV and anogenital cancers, high- and low-grade dysplasia, and genital warts, has led to the development of prophylactic (ie, prior to infection) HPV vaccines. Recent studies in young adult women have shown that prophylactic administration of virus-like particle (VLP) vaccines based on the HPV L1 capsid protein are highly efficacious and immunogenic.[17–22] A phase II, randomized, placebo-controlled study of a quadrivalent HPV-6/

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11/16/18 L1 VLP vaccine included 551 women.[20–22] Through 5.0 years, the overall vaccine efficacy was 96% for preventing HPV 6-, 11-, 16- or 18-related persistent infection and 100% for preventing HPV 6-, 11-, 16- or 18-related disease. In phase III studies conducted in >18,000 young adult women, prophylactic administration of this quadrivalent HPV vaccine was 100% effective in preventing HPV-6, HPV-11, HPV-16 or HPV-18-related precancerous or cancerous lesions of the cervix, vagina and vulva, as well as genital warts through approximately 2 years postvaccination.[18,19] In young adult women, a three-dose regimen of quadrivalent HPV vaccine resulted in robust anti-HPV type-specific immunogenic responses.[20,22] Vaccine-induced antibody responses 1 month following completion of the vaccination series were substantially higher than those observed following natural infection[22]; and in a subset of women with extended follow-up, these responses have been shown to persist (ie, geometric mean titers remained above the seropositivity threshold) through at least 5 years.[21]

If proven safe and effective, a vaccine that prevents cervical cancer, other cervical lesions and genital warts due to HPV-6/11/16/18 will represent a major public health advance. Because the highest risk of acquiring HPV infection is within the 5 years after sexual debut, prophylactic vaccination against HPV should have the greatest benefit in sexually naive adolescents.[23–25] A previous study in male and female adolescents 10 to 15 years of age showed that administration of quadrivalent HPV vaccine is generally well tolerated and produces up to 2-fold higher anti-HPV responses in adolescents compared with young adult women.[26] However, the study was limited by the absence of long-term vaccination follow-up in the adolescent cohort. The present study was designed to assess vaccine safety, to compare the immunogenicity of quadrivalent vaccine in young male versus female adolescents and to explore the safety and duration of immunity of this vaccine for 12 months after the primary series. Unique to this study, the safety comparator for the quadrivalent HPV vaccine was a non–aluminum-containing placebo, whereas all other studies to date have compared the vaccine with aluminum-containing placebo.

METHODS

Study Population

Between October 2003 and March 2004, 1781 healthy, sexually naive boys and girls 9 to 15 years of age were enrolled at 47 study sites located in 10 countries in North America, Latin America, Europe and Asia. Inclusion/exclusion criteria were similar to that described for a noninferiority immunogenicity bridging study.[26] An Institutional Review Board for each clinical site approved the study protocol. At enrollment, written consent was obtained from each participant and his/her legal guardian.

Study Vaccine

The quadrivalent HPV-6/11/16/18 L1 VLP vaccine (GARDASIL/SILGARD, Merck and Co., Inc., Whitehouse Station, NJ) has been described.[22] The placebo used in this study contained identical components to those in the vaccine, with the exception of HPV L1 VLPs and aluminum adjuvant, in a total carrier volume of 0.5 mL. Vaccine and placebo were visually distinguishable.

Study Design

The trial (Merck protocol V501-018) was a randomized, double-blind (with sponsor blinding), placebo-controlled, multicenter study. Enrollment was stratified by age (2:1 ratio of 9- to 12-year-old subjects and 13- to 15-year-old subjects) and by gender (1:1). Randomization schedules were computer-generated using a blocking factor of 6. An interactive voice response system was used to allocate study subjects and to assign allocation numbers. Subjects were randomized in a 2:1 ratio within study centers to receive 3 intramuscular injections of either quadrivalent HPV vaccine or non–aluminum-containing placebo at day 1, month 2 and month 6. The deltoid muscle was the preferred site for intramuscular injection. Vaccine/placebo was administered using a 1.0-mL syringe with needle length of 1 to 2 inches (22–23 gauge).

As the vaccine and placebo used in this study were visually distinguishable, they were prepared and administered by unblinded study personnel not otherwise involved in the care and management of the study participants. The success of blinding was assessed by designated unblinded sponsor and study personnel. To ensure effective monitoring of adverse experiences, an independent safety monitor (not employed by the sponsor) was used. Otherwise, the subject and the investigator, study site personnel, and laboratory personnel conducting the clinical assays were blinded to vaccination group throughout the study. The sponsor's clinical, statistical and data management teams were blinded until the primary analysis at month 7.

A medical history and physical examination were conducted at day 1. If the participant was found to have a temperature of ≥100°F (oral) within 24 hours before an injection, the injection was postponed. For all female subjects, a pregnancy test (sensitive to 25 IU human chorionic gonadotropin) was performed prior to each injection.

Blood samples were obtained on day 1 prior to the first vaccination, month 7 and month 18. Serum specific neutralizing antibodies to HPV-6/11/16/18 were measured using a competitive Luminex immunoassay (cLIA), as described.[27] Seropositivity was defined as anti-HPV serum cLIA levels ≥20, 16, 20 and 24 mMU/mL for HPV-6, HPV-11, HPV-16 and HPV-18, respectively.[27] Seropositivity information was not available prior to the day 1 vaccination. However, if a subject was found to have anti-HPV levels above any serostatus cutoffs at day 1 (prevaccination), indicating prior exposure to one or more vaccine HPV-types, this result was communicated to the primary investigator who had enrolled the subject. After unblinding of the data, investigators were to communicate the finding to the subject and to the parent/legal guardian with appropriate counseling.

Adverse Event Monitoring

Participants were observed for at least 30 minutes after each vaccination for any immediate reaction. Temperatures were recorded orally for 5 days following each injection. All adverse experiences were collected daily by the parent/legal

guardian on a vaccination report card for 14 days following each vaccination. Follow-up at months 2, 6, 7, 12 and 18 included an interview to assess general safety. In addition, at any time during the study, all deaths (regardless of cause) and serious adverse experiences that were considered by the investigator to be vaccine-related were to be reported. The relationship between adverse experiences and vaccine was reported by the investigator according to his/her best clinical judgment, based on exposure, time course, likely cause and probability with vaccine profile.

Statistical Analyses

Safety. The primary safety hypothesis stated that a 3-dose regimen of quadrivalent HPV vaccine is generally well tolerated in adolescents and preadolescents. A detailed tolerability analysis was performed with emphasis on the following prespecified adverse experiences: vaccine-related adverse experiences, injection-site adverse experiences (swelling/redness and pain/tenderness/soreness), systemic adverse experiences (muscle/joint pain, headaches, hives, rashes and diarrhea), severe adverse experiences, and fever. All subjects who received at least one injection and had follow-up data were included in the safety summaries. Adverse experiences were summarized descriptively as frequencies and percentages by vaccination group and type of adverse experience, by vaccination visit and across all vaccination visits. Elevated temperatures (≥100°F oral or oral equivalent) within 5 days following each vaccination were summarized in a similar manner. In addition, risk differences and associated 95% confidence intervals (CI) were computed comparing the vaccine and placebo groups across all vaccination visits with respect to adverse experiences with ≥1% incidence in either vaccination group. *P* values were computed only for those adverse experiences that were prompted for on the vaccination report card (elevated temperatures, injection-site pain, injection-site swelling, injection-site redness, muscle/joint pain, headaches, hives, rashes and diarrhea). Adverse experiences were also summarized separately for boys and girls (within each vaccination group) and by age group. No formal comparisons were made between boys and girls or age groups with respect to adverse experiences.

Immunogenicity

The secondary hypothesis of this study stated that the immune responses to quadrivalent HPV vaccine in preadolescent and adolescent boys are noninferior to the responses in preadolescent and adolescent girls, as measured by anti-HPV GMTs and seroconversion rates one month postdose 3 (month 7). Analyses of noninferiority were conducted based on HPV type-specific per-protocol populations. The per-protocol populations for HPV-6, HPV-11, HPV-16 and HPV-18 consisted of subjects who were seronegative to the relevant HPV type(s) at enrollment, received all 3 doses of vaccine or placebo within the protocol-specified time frames and did not violate the protocol.

The evaluation of noninferiority of boys to girls with respect to the percentage of subjects who seroconverted for each HPV type by month 7 used 4 one-sided tests of noninferiority (one corresponding to each HPV type) conducted at the 0.025 level, based on the methods of Miettinen and Nurminen.[28] To reject the null hypothesis for a given HPV type, the lower bound of the 95% CI on the difference in the percentages of seroconverters (boys minus girls) for that type had to be greater than −5 percentage points.

Noninferiority of boys to girls with respect to month 7 anti-HPV GMTs was tested using an analysis of variance (ANOVA) model. The natural log of the individual titers of the subjects in the quadrivalent HPV vaccine group was modeled as a function of gender, age at enrollment and geographic region, which were considered fixed effects. The analysis was performed using the mean squared error from the ANOVA model as an estimate of variance and a one-sided test for the similarity of 2 means was performed at the 0.025 level. The antilog of the estimated treatment difference in the ANOVA model, and the associated 95% CI, was computed. To reject the null hypothesis for a given HPV type, the lower bound of the 95% CI on the ratio of month 7 GMTs (boys/girls) for that type had to be greater than 0.5.

To declare the immune responses in boys noninferior to those in girls, the statistical criterion had to be met for each HPV type and for each endpoint (month 7 GMTs and seroconversion rates).

A secondary immunogenicity objective was to describe the persistence of immune response to the quadrivalent HPV vaccine. To address this objective, GMTs for each vaccine HPV type, with associated 95% CIs, were summarized at month 18, 1 year following completion of the primary vaccination regimen. In addition, percentages of per-protocol subjects who remained seropositive at month 18 were calculated.

Determination of Sample Size and Power Analysis to Address Study Hypotheses

The primary hypothesis in this study relates to the tolerability of the quadrivalent HPV vaccine. If no vaccine-related serious adverse experiences were observed among 1100 vaccinated subjects, this study provided 95% confidence (one-sided) that the true incidence was no greater than 0.27%. With at least 847, 847, 836 and 836 evaluable subjects in the per-protocol populations related to HPV-6, HPV-11, HPV-16 and HPV-18, respectively (based on predicted rates of attrition and baseline seropositivity rates), the study had >99% power to rule out a ≥2-fold difference in the ratio of GMTs ($\alpha = 0.025$, 1-sided) for each vaccine HPV type and >99% power to rule out a ≥5-percentage-point difference in seroconversion rates between the 2 groups ($\alpha = 0.025$, 1-sided) for each HPV type. If the 2 immunogenicity hypotheses were independent, the overall power of the study to declare noninferiority of immune responses in boys relative to girls was >99%.

The analyses presented here include all safety and immunogenicity data from visits that occurred on or before November 7, 2005. At this time point, the duration of participation was approximately 1.5 years.

Role of the Funding Source

The studies were designed by the sponsor (Merck and Co., Inc.) in collaboration with clinical site investigators. The sponsor collected the data, monitored the conduct of the

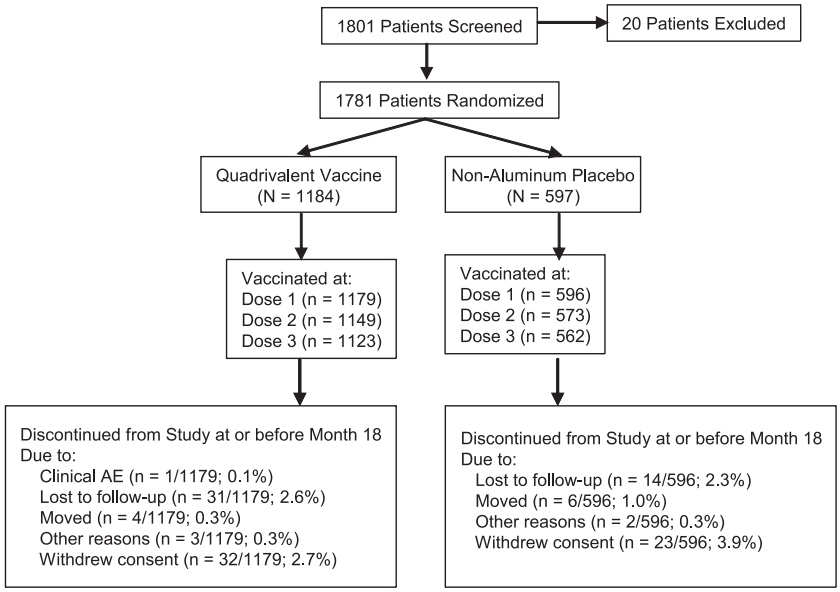

FIGURE 1. Subject disposition flowchart.

study, performed the statistical analysis and coordinated the writing of the manuscript with all authors. Data were unblinded for statistical analyses after the databases were locked. The authors were actively involved in data analysis and interpretation and approved the final manuscript. All authors vouch for the veracity and completeness of the data and the data analyses.

RESULTS

Study Population. Of 1801 subjects screened for eligibility, 1781 were enrolled and randomized to receive quadrivalent vaccine or placebo (Fig. 1). Twenty subjects were screened but not randomized. Of these, 11 withdrew consent prior to randomization, 5 did not meet the inclusion/exclusion criteria, one was unable to provide a sample for the required urine pregnancy test, one was not randomized due to unavailability of the protocol-specified needles, and 2 did not meet the enrollment cutoff for their gender/age group. Of the randomized subjects, 1775 (>99%) received at least one injection. Six subjects withdrew consent and were not vaccinated. A summary of the number of subjects who discontinued from the study, by vaccination group, is provided in Figure 1. The proportions of subjects who failed to complete the vaccination regimen, and the distributions of reasons for discontinuation, were generally similar between the 2 vaccination groups. Lost to follow-up (45 of 1775) and withdrawal of consent (55 of 1775) were the most common reasons for discontinuation.

Key demographic characteristics were generally similar between boys and girls and between subjects who received quadrivalent HPV vaccine or placebo (Table 1). Among all randomized participants, the median age was 12 years and the mean (±standard deviation) body mass index (BMI) was 20.5 (±4.6). The majority of randomized subjects (60.9%)

TABLE 1. Summary of Subject Characteristics by Gender and by Vaccination Group at Enrollment

	Quadrivalent HPV (types 6, 11, 16, 18) L1 VLP Vaccine		Non-Aluminum Placebo		Total (N = 1781)
	Boys (N = 567)	Girls (N = 617)	Boys (N = 275)	Girls (N = 322)	
Age (yr)					
Mean ± SD	12.0 ± 1.9	11.9 ± 1.9	11.8 ± 1.8	11.8 ± 1.9	11.9 ± 1.9
Range	9–16	9–15	9–15	9–15	9–16
Body mass index [weight (kg)/height (m)^2]					
Mean ± SD	20.2 ± 4.4	20.5 ± 4.5	20.3 ± 4.3	21.1 ± 5.1	20.5 ± 4.6
Range	12–41	9–46	14–39	13–51	9–51
Race/ethnicity [no. (%)]					
White	346 (61.0)	370 (60.0)	162 (58.9)	207 (64.3)	1085 (60.9)
Hispanic American	123 (21.7)	137 (22.2)	61 (22.2)	69 (21.4)	390 (21.9)
Asian	67 (11.8)	82 (13.3)	37 (13.5)	33 (10.2)	219 (12.3)
Black	26 (4.6)	24 (3.9)	11 (4.0)	10 (3.1)	71 (4.0)
Native American/Other	5 (0.9)	4 (0.6)	4 (1.5)	3 (0.9)	16 (0.9)
Seropositive to HPV 6, 11, 16, or 18* [no. (%)]	9/555 (1.6)	11/602 (1.8)	4/269 (1.5)	14/314 (4.5)	38/1740 (2.2)

*Numerator = number of subjects who were seropositive at day 1 to one or more of HPV types 6, 11, 16 or 18; denominator = number of subjects with day 1 serum sample.

TABLE 2. Summary of Exclusions From Per-Protocol Immunogenicity Population by Gender

	Quadrivalent HPV (types 6, 11, 16, 18) L1 VLP Vaccine		
	Boys	Girls	Total
No. of subjects included in per-protocol population related to:			
HPV 6	456	492	948
HPV 11	457	492	949
HPV 16	455	489	944
HPV 18	458	494	952
Reasons for exclusion from per-protocol populations*			
General protocol violation	57	72	129
Missing or invalid day 1 serology sample/results			
HPV 6	10	14	24
HPV 11	10	14	24
HPV 16	10	15	25
HPV 18	9	14	23
Missing or invalid month 7 serology sample/results			
HPV 6	24	19	43
HPV 11	23	19	42
HPV 16	25	22	47
HPV 18	26	19	45
Month 7 serology sample out of acceptable day range	28	30	58
Day 1 seropositive to HPV 6 or 11†	4	5	9
Day 1 seropositive to HPV 16†	4	4	8
Day 1 seropositive to HPV 18†	1	2	3

*A subject may appear in more than one category; however, a subject is counted only once in the total number excluded.
†Exclusion due to day 1 seropositivity applies only to respective per-protocol population.

were white, followed by Hispanic American (21.9%). Day 1 anti-HPV titers above the seropositivity cutoff for a given HPV type (indicative of previous exposure to that type) were detected in 38 of 1740 (2.2%) of subjects. Sixteen subjects were positive to HPV-6, 2 were positive to HPV-11, 18 were positive to HPV-16 and 5 were positive to HPV-18. Among these baseline seropositive subjects, 66% were girls and 34% were boys.

Immunogenicity. The immune response generated by a 3-dose regimen of quadrivalent HPV vaccine was compared between boys and girls. Analyses of noninferiority were conducted in the HPV-6, HPV-11, HPV-16 and HPV-18 per-protocol cohorts, which included 948, 949, 944, and 952 quadrivalent vaccine recipients, respectively (Table 2). Approximately 80% of the study participants met the protocol's criteria for inclusion in the per-protocol evaluation (Table 2). The most common reason for exclusion was a general protocol violation (129 subjects), such as an incomplete vaccination series or vaccination outside of an acceptable day range. The proportion of girls excluded from the per-protocol analysis of each vaccine HPV type was slightly higher (19.6–20.4%) than the proportion of boys excluded (18.7–19.2%).

For each of the 4 vaccine types, ≥99.5% of subjects in the respective per-protocol immunogenicity cohort had seroconverted by 1 month after completion of the 3-dose regimen, regardless of gender (Table 3). The lower bound of the 95% CI for the difference (boys minus girls) in seroconversion rates was ≤−5% points for each vaccine type ($P < 0.001$ for each vaccine component).

TABLE 3. Per-Protocol Analyses of Month 7 Anti-HPV Responses

Parameter	Boys		Girls		Difference/Fold Difference (95% CI)*
	n	Response	n	Response	
Anti-HPV 6					
% Seroconversion	456	99.8	492	99.8	0.0 (−1.0, 1.0)
GMT (mMU/mL)		1007		808	1.3 (1.0, 1.5)
Anti-HPV 11					
% Seroconversion	457	99.8	492	99.8	0.0 (−1.0, 1.0)
GMT (mMU/mL)		1334		1187	1.1 (0.9, 1.4)
Anti-HPV 16					
% Seroconversion	455	99.5	489	99.8	−0.2 (−1.4, 0.8)
GMT (mMU/mL)		6316		4490	1.4 (1.1, 1.8)
Anti-HPV 18					
% Seroconversion	458	99.8	494	99.6	0.2 (−0.8, 1.3)
GMT (mMU/mL)		1581		1071	1.5 (1.2, 1.9)

*Difference = Boys minus girls; Fold difference = boys divided by girls. $P < 0.001$ for all tests of noninferiority of immune responses in boys to those in girls (for all 4 vaccine HPV types for both endpoints).

TABLE 4. Per-Protocol Summary of Month 18 Anti-HPV Responses

Parameter	Boys			Girls		
	n	Response	95% CI	n	Response	95% CI
Anti-HPV 6						
% Seroconversion	449	97.8	(95.9, 98.9)	481	97.9	(96.2, 99.0)
GMT (mMU/mL)		227	(204, 251)		213	(195, 232)
Anti-HPV 11						
% Seroconversion	450	99.3	(98.1, 99.9)	481	99.2	(97.9, 99.8)
GMT (mMU/mL)		292	(263, 323)		300	(273, 330)
Anti-HPV 16						
% Seroconversion	448	99.3	(98.1, 99.9)	478	99.8	(98.8, 100)
GMT (mMU/mL)		1,402	(1252, 1570)		1,250	(1134, 1378)
Anti-HPV 18						
% Seroconversion	451	92.5	(89.6, 94.7)	483	91.5	(88.7, 93.8)
GMT (mMU/mL)		233	(201, 270)		181	(159, 205)

Table 3 displays the results of the analysis of noninferiority with respect to GMTs as measured 1-month postcompletion of the vaccination regimen. The table displays the GMTs, along with the fold differences in GMTs (boys divided by girls), and the 95% CIs on the fold differences. The lower bound of the 95% CI on the fold difference exceeded 0.5 for each vaccine HPV type, thus supporting the conclusion that the anti-HPV GMTs in boys are noninferior to those in girls ($P < 0.001$ for each vaccine component). Of note, for all vaccine types, numerically higher GMTs were observed in boys compared with girls.

Baseline characteristics were evaluated for their potential to affect anti-HPV responses. The magnitude of vaccine-induced anti-HPV responses varied with age at first vaccination. At 1-month postdose 3, anti-HPV-6, anti-HPV-11, anti-HPV-16 and anti-HPV-18 GMTs were approximately 1.4-, 1.5-, 1.5- and 1.6-fold higher, respectively, in subjects who were 9 to 12 years old compared with subjects who were 13 to 15 years old at first vaccination. A number of vaccines have been shown to produce suboptimal immune responses if administered subcutaneously, and for the very overweight, many IM injections end up subcutaneous. In general, anti-HPV responses for HPV types 6 and 11 did not appear to be affected by BMI. Subjects with BMIs less than 28 had generally comparable GMTs to subjects with BMIs greater than or equal to 28. The greatest apparent effect of BMI on anti-HPV responses was observed with respect to HPV-16 and HPV-18, for which girls with a BMI ≥28 (GMT = 2531, 95% CI 1586 to 4040 for HPV-16 and GMT = 538, 95% CI 320 to 903 for HPV-18) appeared to have lower responses than girls with a BMI <28 (GMT = 5195, 95% CI 4654 to 5800 for HPV-16 and GMT = 1182, 95% CI 1065 to 1313 for HPV-18).

A secondary immunogenicity objective of the study was to describe the persistence of the immune response. One year postcompletion of the vaccination regimen (month 18), ≥91.5% of all vaccine recipients in the per-protocol population remained seropositive, regardless of gender (Table 4). In both boys and girls, GMTs at month 18 were approximately 4- to 7-fold lower than the GMTs observed at month 7.

Safety. Adverse experiences were common among both vaccine and placebo recipients. Table 5 summarizes the observed adverse experiences after each dose and across all vaccination visits. The proportion of subjects who reported one or more injection-site or systemic adverse experience tended to be higher after the first injection than after subsequent injections, regardless of vaccination group.

A comparison of the vaccine and placebo groups across all vaccination visits showed that a significantly higher proportion of subjects in the quadrivalent HPV vaccine group reported injection-site adverse experiences days 1 to 5 after any vaccination than in the non–aluminum-containing placebo group (Table 5). Significantly higher percentages of subjects in the quadrivalent HPV vaccine group reported injection-site erythema, pain and swelling days 1 to 5 across all vaccination visits compared with the non–aluminum-containing placebo group ($P < 0.001$).

The most common systemic adverse experiences reported were headache, fever and pharyngeal pain. There was no significant difference between vaccination groups with regard to the proportion of subjects who reported specific systemic adverse experiences prompted for on the vaccination report card (muscle/joint pain, headaches, rashes, hives, and diarrhea) days 1 to 15 across all vaccination visits.

The proportion of subjects who reported an elevated temperature within 5 days across all vaccination visits was not significantly different between subjects who received the quadrivalent HPV vaccine compared with subjects who received placebo (7.2 vs. 6.6% $P = 0.638$; Table 5). Most fevers were low grade (maximum temperature below 102°F or 38.9°C).

Overall, 5 serious adverse experiences were reported through month 18, all of which occurred among the quadrivalent HPV vaccine recipients. None of these serious adverse experiences was judged by the investigator to be vaccine-related. Two of these serious adverse experiences occurred 6 days and 2 days, respectively, after the first injection: acute renal failure (subject recovered and discontinued from study); and insulin dependent diabetes mellitus. Three occurred 2 days, 11 days and 3 days, respectively, after the second injection: localized infection; anemia and dysfunctional uterine bleeding; and appendicitis. Two subjects in the quadrivalent HPV vaccine group and none in the placebo group discontinued treatment due to a nonserious vaccine-related

TABLE 5. Adverse Experience Summary Days 1–15 Postdose 1, 2 and 3 and Across All Vaccinations

	Postdose 1		Postdose 2		Postdose 3		Across All Vaccinations	
	Vaccine	Non-Aluminum Placebo	Vaccine	Non-Aluminum Placebo	Vaccine	Non-Aluminum Placebo	Vaccine	Non-Aluminum Placebo
Subjects with follow-up	1165	584	1139	564	1120	559	1165	584
No. (%)* of subjects								
With 1 or more AE	779 (66.9)	312 (53.4)	627 (55.0)	200 (35.5)	577 (51.5)	191 (34.2)	963 (82.7)	392 (67.1)
Injection-site AE	663 (56.9)	198 (33.9)	555 (48.7)	131 (23.2)	517 (46.2)	137 (24.5)	877 (75.3)	292 (50.0)
Erythema†	91 (7.8)	42 (7.2)	105 (9.2)	31 (5.5)	123 (11.0)	30 (5.4)	237 (20.3)	77 (13.2)‡
Pain†	623 (53.5)	180 (30.8)	532 (46.7)	114 (20.2)	494 (44.1)	124 (22.2)	853 (73.2)	265 (45.4)‡
Swelling†	91 (7.8)	27 (4.6)	106 (9.3)	13 (2.3)	135 (12.1)	19 (3.4)	241 (20.7)	45 (7.7)‡
Systemic AE	377 (32.4)	199 (34.1)	202 (17.7)	97 (17.2)	168 (15.0)	84 (15.0)	541 (46.4)	260 (44.5)
With serious AE	2 (0.2)	0 (0.0)	3 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	5 (0.4)	0 (0.0)
With serious vaccine-related AE	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fever								
Subjects with follow-up	1153	574	1121	554	1105	552	1157	579
<100° F or normal†	1122 (97.3)	557 (97.0)	1092 (97.4)	540 (97.5)	1075 (97.3)	538 (97.5)	1074 (92.8)	541 (93.4)
≥100° F†	31 (2.7)	17 (3.0)	29 (2.6)	14 (2.5)	30 (2.7)	14 (2.5)	83 (7.2)	38 (6.6)§

*Percentages are calculated based on the number of subjects with follow-up.
†Adverse experiences reported days 1 to 5 following any vaccination visit.
‡$P < 0.001$, for comparison of vaccination groups across all vaccination visits.
§$P = 0.638$, for comparison of vaccination groups across all vaccination visits.
AE indicates adverse experience.

adverse experience. The reasons were injection-site swelling, whereby the subject discontinued treatment after receiving the second dose of quadrivalent vaccine; and injection-site pain, whereby the subject discontinued treatment after receiving the first dose of quadrivalent vaccine. Both subjects continued in the study for follow-up only. Through month 18, the proportions of subjects reporting new medical conditions were comparable between the 2 vaccination groups. In both groups, the most common new condition was influenza.

Regardless of vaccination group, a higher proportion of girls than boys reported adverse experiences, although no formal comparisons between genders were performed. The adverse experience findings in boys versus girls were generally comparable to those observed when considering subjects by vaccination group.

DISCUSSION

Administration of a quadrivalent HPV vaccine to 9- to 15-year-olds was generally safe and well tolerated. A larger proportion of subjects who received the quadrivalent vaccine experienced injection-site adverse experiences compared with placebo subjects. However, few subjects discontinued vaccination because of an adverse experience. In this age group, the quadrivalent HPV vaccine was highly immunogenic and persistent immune responses were observed through 1-year postdose 3. In this study, and in a previous study,[26] the magnitude of anti-HPV responses varied with age at first vaccination, with the younger cohort having the most robust vaccine-induced anti-HPV responses. Vaccine-induced responses in 9- to 15-year olds were substantially higher than the vaccine-induced responses observed in 16- to 23-year-old women, the age group in whom 100% prophylactic efficacy of the vaccine has been demonstrated.[18,19,22] In young women, the efficacy and immunogenicity of this quadrivalent vaccine have been demonstrated to persist through at least 5 years.[21] Thus, administration of quadrivalent HPV vaccine to young adolescents should similarly induce protective efficacy.

An expected drop in anti-HPV responses was observed between month 7 (1-month postdose 3) and month 18. In women aged 16 to 23, vaccine-induced anti-HPV responses have been shown to decline postvaccination, plateau between months 18 and 24 and remain stable through 5 years.[20,21] Additional data are needed to determine if the anti-HPV responses in 9- to 15-year olds plateau in a similar manner. There is no known immune correlate of protection for HPV. The vaccine-induced immune response appears highest for HPV-16; however, direct comparisons of the relative immunogenicity of the 4 VLP components cannot be made from the absolute titers, as the titers for each of the reference sera for the individual assays are not identical. The serology assays used in these studies measured HPV antibody titers in a competitive format whereby serum antibodies compete with HPV type-specific mouse mAbs to neutralizing epitopes present on each VLP.[20] The scale of the competitive immune response is dependent upon the particular attributes of the mAbs and the epitope that they recognize.

Ideally, prophylactic vaccines should be administered to populations immediately before their entry into the period of greatest risk for acquisition of the infection targeted by the vaccine. This principle must take into account the available data regarding the length of the risk period and the known duration of protection of the vaccine. Implementation of public health policy related to HPV vaccination must address 2 fundamental issues: 1) the optimal age for vaccination; and 2) whether vaccination should be limited to girls and women, or offered to both genders. The first 5 to 10 years following sexual debut represent the period of highest risk for acquisition of HPV infection.[8–10] In many countries, most adolescents will have experienced sexual debut by age 16 years. In

the United States, 7.9% of 14-year-old students participating in a population-based survey reported having already experienced sexual debut.[11] Thus, prophylactic HPV vaccination campaigns should be initiated before this age. While implementation of HPV vaccination campaigns in adolescents is reasonable, limited information regarding the safety, immunogenicity and duration of efficacy of quadrivalent HPV vaccine in this age group has been available. The results of the current study show that the vaccine is durable for up to 12 months postdose 3.

The question of whether HPV vaccination should be administered to girls and women, or to the population as a whole must be considered in the context of the epidemiology of HPV disease and previous experiences with implementation of new vaccines. HPV infection in men is common. Men are the primary vector for transmission of HPV to women. Over 10% of men will acquire a case of detectable genital warts during their lifetime.[5] Among men having sex with men, the incidence of anal cancer approaches the incidence observed for cervical cancer in settings where Pap testing is not routinely available.[29] Thus, men could derive significant benefit from HPV vaccination.

The optimal population benefit of vaccination is achieved through induction of herd immunity, defined as the induction of protective immunity in a sufficient proportion of the population such that unvaccinated subjects are protected because they are not exposed to the pathogen targeted by the vaccine.[30] The experience of rubella vaccination for the prevention of congenital rubella syndrome (CRS) in the United Kingdom demonstrated the value of universal vaccination to prevent serious infection-related diseases that affects only one gender. While both men and women can be infected by rubella, CRS can only occur as a consequence of infection in women because CRS is caused by infection of the fetus in utero. Rubella vaccination programs in the United Kingdom initially targeted girls only but was later changed to gender-neutral vaccination. This transition occurred because it was not possible to vaccinate 100% of women, the large reservoir of infected men precluded the development of herd immunity, and epidemics of infection in young adults invariably led to infection of pregnant women and spikes in the incidence of CRS. The eradication of CRS in the United Kingdom occurred only after a universal vaccination policy was adopted. HPV is highly prevalent in the sexually active population. Thus, universal vaccination is highly likely to lead to a more rapid reduction in the burden of HPV disease than gender-specific vaccination.

Together, these factors suggest that HPV vaccination programs should be gender neutral. However, the efficacy of prophylactic HPV vaccines in boys and men has yet to be determined. The results of the current study provide additional information on the safety and immunogenicity of the quadrivalent HPV vaccine in boys. In the study, vaccine-induced anti-HPV levels in boys were noninferior (and, in fact, numerically superior) to those observed in girls. The adverse experience profiles in boys and girls were comparable. The similar GMTs and seroconversion of rates of boys compared with girls and 16- to 23-year-old women[26] suggests that prophylactic HPV vaccination will induce durable protective efficacy in boys and men. A formal efficacy study is ongoing to definitively evaluate vaccine efficacy in men with respect to genital warts, penile and anal cancer.

CONCLUSION

The current study demonstrates that administration of quadrivalent HPV-6/11/16/18 L1 VLP vaccine to 9- to 15-year-old boys and girls is highly immunogenic, provides durable immunity through 1 year postvaccination and is generally well tolerated. These results further support the implementation of gender neutral HPV vaccination program to eradicate cancers, precancerous lesions and genital warts caused by vaccine HPV types.

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