ARTICLE

Comparison of the Immunogenicity and Reactogenicity of a Prophylactic Quadrivalent Human Papillomavirus (Types 6, 11, 16, and 18) L1 Virus-Like Particle Vaccine in Male and Female Adolescents and Young Adult Women

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ABSTRACT

OBJECTIVE. Prophylactic vaccination of 16- to 23-year-old females with a quadrivalent human papillomavirus (types 6, 11, 16, 18) L1 virus-like particle vaccine has been shown to prevent type-specific human papillomavirus infection and associated clinical disease. We conducted a noninferiority immunogenicity study to bridge the efficacy findings in young women to preadolescent and adolescent girls and boys, who represent a primary target for human papillomavirus vaccination.

METHODS. We enrolled 506 girls and 510 boys (10–15 years of age) and 513 females (16–23 years of age). Participants were vaccinated on day 1, at month 2, and at month 6, and serology testing was performed on day 1 and at months 3 and 7 on blinded samples. Neutralizing antibody concentrations were determined using type-specific immunoassays and summarized as geometric mean titers and seroconversion rates. Vaccine tolerability also was assessed.

RESULTS. By month 7, seroconversion rates were ≥99% for all 4 human papillomavirus types in each group. By month 7, compared with women, anti–human papilloma virus geometric mean titers in girls or boys were noninferior and were 1.7- to 2.7-fold higher. Most (>97%) injection-site adverse events were mild to moderate in intensity. Significantly more boys (13.8%) and girls (12.8%) than women (7.3%) reported fevers ≥37.8°C within 5 days of vaccination. Most (96.4%) fevers were mild (<39°C).

CONCLUSIONS. Noninferior immunogenic responses to all 4 human papillomavirus types in the quadrivalent vaccine permit the bridging of efficacy data that were generated in young women to girls. The results in boys lend support for the implementation of gender-neutral human papillomavirus vaccination programs. This vaccine generally was well tolerated.

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

Abbreviations
HPV— human papillomavirus
VLP—virus-like particle
PCR—polymerase chain reaction
cLIA— competitive Luminex xMAP-based immunoassay
mMU—milliMerck unit
VRC—vaccination report card
AE—adverse event
GMT— geometric mean titer
PPI—per-protocol immunogenicity
CI— confidence interval
SIL—squamous intraepithelial lesion

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ANOGENITAL HUMAN PAPILLOMAVIRUS (HPV) infection is the most common viral infection of the anogenital tract worldwide, with a cumulative lifetime risk for infection approaching 70%.[1] HPV infection in the anogenital tract is often cleared spontaneously without clinical sequelae; however, if not cleared, infection can lead to clinical disease that ranges from early mild dysplasia or genital warts to severe dysplasia and cervical cancer.[2,3] Worldwide, >500 000 cases of cervical and other genital cancers are caused by HPV infection annually with >273 000 deaths attributable to cervical cancer.[4] The lifetime risk for acquired genital warts exceeds 10%.[5]

The HPV family includes ~100 types, 35 to 40 of which infect the epithelium of the anogenital tract. All genital HPV types can cause dysplasia and visible lesions. However, only a subgroup of HPV types cause dysplasia that can lead to cancer. HPVs 16 and 18 cause ~70% of cervical and other HPV-related genital cancers. They also are the causative agents for ~25% to 35% and 50% to 70%, respectively, of low-grade and high-grade cervical dysplastic lesions.[1] HPVs 6 and 11 cause ~90% of all cases of classic condyloma acuminata (genital warts) as well as 10% to 15% of low-grade cervical dysplastic lesions, both of which rarely cause cancer,[6] and >90% of juvenile onset recurrent respiratory papillomatosis.[7]

Recombinant synthesis of the HPV L1 protein, the major constituent of the viral capsid, results in assembly of virus-like particles (VLPs), which, when administered with adjuvant, are potent immunogens, inducing immune responses that are higher than that seen after natural infection.[8] In a recent placebo-controlled study of 551 16- to 23-year-old females, a prophylactic (ie, before infection) 3-dose regimen of a quadrivalent HPV (types 6, 11, 16, and 18) VLP L1 vaccine resulted in robust anti–HPV specific neutralizing antibody responses that led to a 90% reduction in the combined incidence of HPV type–specific persistent infection or cervical or external genital disease.[9] In a subsequent study of this vaccine in >12 000 women aged 16 to 26 years, similar robust immune responses were observed, leading to 100% prevention of HPV 16– and HPV 18–related cervical intraepithelial neoplasia grades 2/3 and adenocarcinoma in situ and pre- or noninvasive cervical cancer, compared with placebo.[10] These findings suggested that vaccination of individuals who are not yet infected with HPV will reduce substantially their risk for developing cervical cancer and genital warts later in life. The highest risk for acquiring HPV infection is within the first 5 years after sexual debut. Therefore, an HPV vaccine ideally should be administered to children who are aged 15 or younger, at a time before the high-risk period after sexual debut.[11–14]

Studies to demonstrate the efficacy of prophylactic HPV vaccines in preadolescents and adolescents are not feasible given legal and ethical issues regarding evaluations of sexual activity in this population and the relatively low rate of exposure at this age. Therefore, efficacy studies of these vaccines have not been conducted in adolescents who are younger than 16 years. To bridge the demonstrated vaccine efficacy findings in 16- to 23-year-old females to preadolescents and adolescents, who represent a primary target for HPV vaccination, we conducted an immunogenicity substudy within a larger dose-response study (for gathering vaccine product expiration dating information). The objective of this immunogenicity substudy was to determine whether HPV L1 VLP vaccine-induced anti-HPV neutralizing antibody responses in 10- to 15-year-old girls and boys are comparable to responses in 16- to 23-year-old females.[9,15]

METHODS

Participants

Sixty-one clinical centers (academic medical centers and private practice–based research centers) in Asia, Australia, Europe, Latin America, and North America participated in this clinical study between December 7, 2002, and September 20, 2004. The institutional review board or local ethics committee of each study center approved this protocol. Parents of all study participants who were younger than 18 years provided written informed consent, and, when required, these study participants also provided written informed assent. All adult study participants provided written informed consent before their participation in the study.

The study was designed to enroll 3 populations: 10- to 15-year-old girls, 10- to 15-year-old boys, and 16- to 23-year-old females. For the 2 younger populations, participants were required to be sexually naïve at enrollment and throughout the study and to be generally healthy. For the older population, participants were required to be generally healthy and have an intact uterus, no evidence of gross purulent cervicitis, no history of genital warts, no previous abnormal Papanicolaou tests, no history of cervical intraepithelial neoplasia, and a lifetime history of no more than 4 sexual partners.

Individuals were excluded from participation when they were pregnant (determined by urine or serum β-human chorionic gonadotropin testing), were allergic to any vaccine component, had received any blood product or component in the previous 6 months, had any known immune or coagulation disorder, or had received any inactivated vaccine product within 14 days before enrollment or any live vaccine product within 21 days before enrollment.

Vaccine Preparation and Administration

A description of the quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP vaccine was reported previously.[9] Briefly, the vaccine consists of a mixture of 4 recombinant HPV type–specific VLPs (Merck Research Laborato-

ries, West Point, PA) that consist of the L1 major capsid proteins of HPVs 6, 11, 16, and 18, each of which were synthesized in *Saccharomyces cerevisiae*.[15–17] The 4 VLP types were adsorbed onto amorphous aluminum hydroxyphosphate sulfate adjuvant after purification. In addition to containing 225 μg of aluminum adjuvant, each 0.5-mL dose of the vaccine test product contained the following amounts of L1 VLP: 20 μg of HPV 6, 40 μg of HPV 11, 40 μg of HPV 16, and 20 μg of HPV 18. The appropriate concentrations of HPV type–specific L1 VLP antigens were determined from the results of a previous study.[9] The vaccine was administered by intramuscular injection (0.5 mL) into the upper arm or thigh.

Study Design

The trial (sponsor protocol no. V501-016) was an age- and gender-stratified noninferiority immunogenicity study with a target enrollment of ~500 participants in each study cohort. It was designed to compare the immune response to each HPV type after a 3-injection regimen of a full dose of a quadrivalent HPV L1 VLP vaccine through month 7. The 10- to 15-year-old participants also were followed for 1 year after the initial vaccination visit to collect additional safety-related data. This study was a substudy within a randomized, double-blinded, multi-dose study that was designed to obtain data that were needed to determine specifications for vaccine product expiration dating. It examined the immunogenic response of lower vaccine doses (20%, 40%, and 60%) in girls and women compared with the full dose (100%) reported here (Merck & Co, Inc, data on file). Randomization was with respect to the multidose study. With respect to the noninferiority comparison of immunogenic responses between adults and adolescents reported here, the study was not blinded or randomized; however, all biological samples were coded to maintain analyst blinding. All participants in this noninferiority immunogenicity study received the full dose of vaccine.

Participants received a full dose of vaccine on day 1, at month 2 (±3 weeks), and at month 6 (±3 weeks). All participants were required to be afebrile (oral temperature <37.8°C) within 24 hours before each injection. All female participants underwent pregnancy testing that was based on urine or serum analyses for β-human chorionic gonadotropin before each vaccination. Participants who were found to be pregnant were not vaccinated.

The older female group was instructed to use effective contraception through month 7 of the study and refrain from sexual activity or use of vaginal medications or cleansing products within 48 hours of any scheduled clinic visit that included a pelvic examination. Only female participants in the older age group underwent Papanicolaou testing. Participants with abnormal Papanicolaou tests at day 1 were followed up as per the local standard of care. External genital and cervical swabs were obtained for polymerase chain reaction (PCR) detection of HPVs 6, 11, 16, or 18 DNA, indicative of infection, in the 16- to 23-year-old participants on day 1 and at month 7. HPVs 6, 11, 16, and 18 DNA were detected using fluorescence-based multiplex PCR assays that were designed to detect DNA from the L1, E6, and E7 genes of each virus.

Immunogenicity

Serum samples were obtained at day 1, at month 3, and at month 7 in all participants. Samples were stored at −20°C or below until testing. Anti-HPV levels were determined using an HPV type–specific competitive Luminex xMAP-based immunoassay (cLIA), as previously described.[18,19] This assay measures only neutralizing anti-HPV antibodies, rather than the broad assortment of vaccine-induced anti-HPV antibodies (some of which are not neutralizing). Antibody levels were expressed as milliMerck units (mMU) per milliliter. The scales for each type-specific assay were set using standards from vaccinated primates. Each scale was set separately; therefore, cross-assay comparisons of anti-HPV levels are not valid. The lower limits of detection for the anti-HPV 6, 11, 16, and 18 cLIAs were 4.1 mMU6/mL, 3.0 mMU11/mL, 10.2 mMU16/mL, and 2.9 mMU18/mL, respectively. Assay precision was estimated to be 21.7%, 20.4%, 23.0%, and 15.9% for the anti-HPV 6, 11, 16, and 18 cLIAs respectively. Participants were considered anti-HPV 6, 11, 16, or 18 seropositive when their anti-HPV antibody titers were ≥20 mMU6/mL, 16 mMU11/mL, 20 mMU16/mL, or 24 mMU18/mL, respectively.

Reactogenicity and Overall Vaccine Safety

All participants were observed for at least 30 minutes after each vaccination for any immediate reaction, with particular attention to any evidence of a hypersensitivity reaction. Adult participants recorded their oral temperatures 4 hours after each vaccine injection and daily for the next 4 days on a vaccination report card (VRC). The VRCs for 10- to 15-year-olds were completed by a parent or legal guardian. Any systemic or local adverse events (AEs) that occurred within 14 days of a vaccine injection were recorded on each participant's VRC. Participant-reported AEs also were collected at months 2, 3, and 7 using an interview process. Investigators were instructed to assign causality to AEs on the basis of exposure, time course, likely cause, and consistency with the test vaccine's known profile. Vaccine-related AEs were those that were determined by the investigator to be possibly, probably, or definitely vaccine related. For each AE, participants were asked to rate the symptom as mild (awareness of sign or symptom but easily tolerated), moderate (discomfort enough to cause interference with usual activities), or severe (incapacitating with inability to work or do usual activity). Serious AEs were predefined as any AEs that resulted in death, were deemed

by the investigator to be life-threatening, or resulted in a persistent or significant disability or incapacity. An independent safety monitor was put in place to monitor participant safety continuously because this was the first study of this quadrivalent vaccine in 10- to 15-year-olds.

Statistical Analysis

Immunogenicity

The primary hypothesis of this study was that the immune responses to the quadrivalent HPV (types 6, 11 16, and 18) L1 VLP vaccine in 10- to 15-year-old boys or girls are noninferior to the immune responses that are observed in 16- to 23-year-old females. The end points of the study were the geometric mean titers (GMTs) of neutralizing antibodies for each HPV type at week 4 postdose 3 (month 7) and the percentages of participants who seroconverted to each HPV type by month 7. To demonstrate noninferiority of the quadrivalent HPV L1 VLP vaccine's immunogenicity in either boys or girls compared with young adult women, noninferiority had to be met for both primary end points and for each of the 4 HPV vaccine types. The sample size for the study was chosen to provide >99% power to declare noninferiority in immunogenic responses and seroconversion rates for at least 1 of the populations (girls or boys compared with women). A step-up multiplicity procedure was used to control the overall type I error rate $\alpha = .025$ (1 sided) with respect to the 2 demographic group comparisons.

The primary analysis approach was per-protocol. To be included in the per-protocol immunogenicity (PPI) population for each HPV type, participants were required to have received 3 appropriate doses of vaccine within prespecified visit intervals, to have had the month 7 serum sample drawn within a prespecified interval relative to dose 3, not to have violated the protocol in ways that potentially would interfere with the vaccine's immunogenicity, and to be seronegative to the respective HPV type at day 1. Because the L1 proteins for HPV 6 and HPV 11 are 92% homologous at the amino acid level, all participants were required to be both anti–HPV 6 and anti–HPV 11 seronegative to be included in either of the HPV 6 and HPV 11 PPI populations. Note that because there were differing numbers of participants who were seropositive to the different HPV types at day 1, the numbers of participants in the per-protocol study populations are type specific. Only 16- to 23-year-olds were required to undergo genital sampling for PCR detection of HPV. They were required to be PCR negative to the respective vaccine types throughout the course of the study. Confirmatory analyses of antibody responses in participants who were naïve to all HPV types and had immunogenicity data also were conducted. This population included participants who were not included in the per-protocol analysis (eg, because of protocol violations).

Confirmatory analyses of antibody responses in participants who were naïve to the respective HPV types and had immunogenicity data also were conducted. This population included participants, regardless of general protocol violations, who received 3 appropriate doses of vaccine and remained PCR negative to the respective vaccine types throughout the course of the study.

To address the primary hypothesis of noninferiority, the immune responses of girls and boys were compared separately with those of women. Noninferiority with respect to anti-HPV GMTs was addressed by 4 1-sided tests of noninferiority (1 for each HPV vaccine type) conducted at the 0.025 level (multiplicity adjusted). Each test was addressed by analysis of variance, modeling the postdose 3 natural log of the anti-HPV cLIA value as a function of geographic region, demographic group (girls, boys, or women), and interaction of demographic group by geographic region (all modeled as fixed effects). The fold difference in GMTs (girls/women, boys/women) was computed as the anti-log of the estimated group difference in the analysis of variance model along with its associated 95% confidence interval (CI). For noninferiority of GMTs to be declared in girls or boys versus women, the lower bound of the multiplicity-adjusted 95% CIs for the GMT ratios (girls/women, boys/women) had to be >0.5 for all HPV types. This lower confidence bound was chosen to be consistent with other trials in the quadrivalent HPV vaccine program and based on regulatory guidance and clinical judgment regarding clinically meaningful differences in immune responses.

To test for noninferiority of seroconversion rates in boys or girls to those in women, we conducted 4 1-sided tests of noninferiority (1 corresponding to each HPV type) at the 0.025 level (multiplicity adjusted). These tests were conducted using the method of Miettinen and Nurminen,[20] which allows stratification by region. When the lower bounds of the multiplicity-adjusted 95% CIs for the differences in seroconversion rates (% boys or girls − % women) were −5 percentage points or more for all HPV types, noninferiority was concluded.

Safety

All participants who received at least 1 injection and had follow-up data were included in the safety analysis. AEs were summarized descriptively as frequencies and percentages by participant group and type of AE, by vaccination visit, and across all vaccination visits. In addition, risk differences and associated 95% CIs were computed comparing girls and boys separately with women across all vaccination visits with respect to AEs with an incidence ≥1% in either group being compared. Elevated temperatures (≥37.8°C, oral equivalent) within 5 days after each vaccination were summarized in a similar

manner. *P* values were computed for group differences in elevated temperatures and reactogenicity (injection-site pain, swelling, and redness). Pairwise comparisons of prespecified AEs and elevated temperatures between boys and women and between girls and women were made using the method of Miettinen and Nurminen[20] at a 2-sided $\alpha = .05$ significance level. No adjustments for multiple comparisons were made for safety analyses.

RESULTS

Demographics

A total of 1529 participants were enrolled in this bridging substudy (Fig 1), and 1525 participants received at least 1 injection of the vaccine. Of those participants, 1450 (94.8%) completed the vaccination regimen. Loss to follow-up (2.4%) and withdrawal of consent (1.9%) were the 2 most frequent reasons for discontinuation (Fig 1).

Both adolescent groups generally were well balanced with regard to baseline demographic characteristics (age, weight, and race/ethnicity) except for geographic region (Table 1). As expected, the population of 16- to 23-year-olds on average was heavier and had somewhat larger BMIs than the younger age groups. Among the 16- to 23-year-old females, 88% were nonvirgins with a mean (SD) age of first sexual intercourse of 17 (2) years. The median number of lifetime male or female sexual partners in this older age group was 2, and 19.5% of participants reported a previous pregnancy. Of 16- to 23-year-olds who had a Papanicolaou test result from day 1 ($n = 509$), 41 (8.1%) had a diagnosis suggestive of a squamous intraepithelial lesion (SIL), which included 19 (3.8%) atypical squamous cells of undetermined significance; 3 (0.6%) atypical squamous cells of undetermined significance, cannot rule out high-grade SIL; and 19 (3.8%) low-grade SIL. All 3 groups were well balanced with respect to race and ethnicity.

At enrollment, serum antibody concentrations for HPV 6, 11, 16, or 18 that were greater than the predefined seropositive threshold values for 1 or more HPV type, indicative of previous exposure to the respective

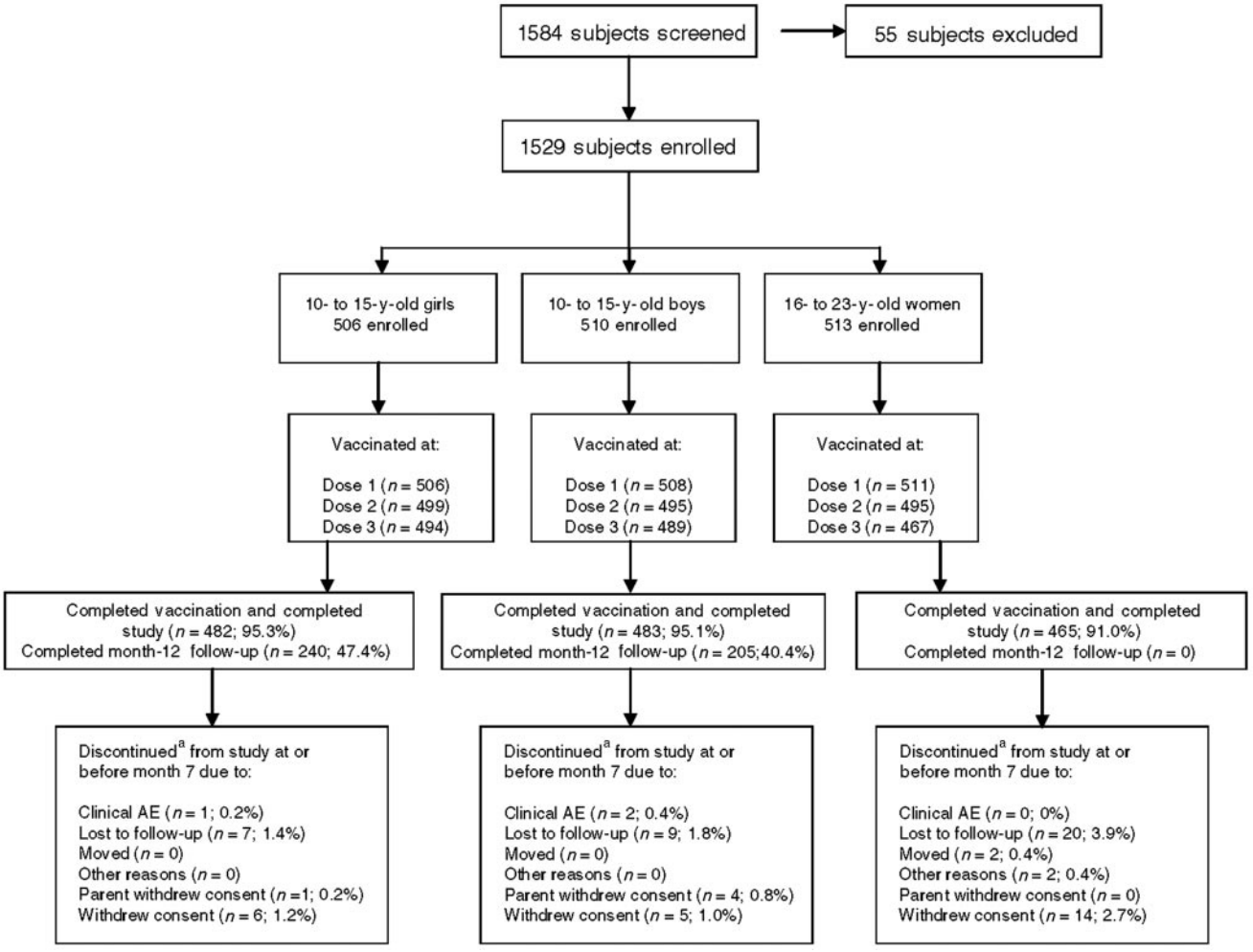

FIGURE 1
Participant disposition flowchart. [a] Nine girls and 3 boys were lost to follow-up after month 7. Two boys and 8 women discontinued the vaccinations but completed the study.

TABLE 1 **Baseline Demographics of Enrolled Study Participants**

	Girls (10–15 y old; $N = 506$)	Boys (10–15 y old; $N = 510$)	Women (16–23 y old; $N = 513$)
Age, mean (SD), y	12.6 (1.6)	12.6 (1.6)	20.0 (2.1)
Weight, mean (SD), kg	50.8 (15.3)	53.1 (17.1)	60.6 (12.9)
BMI, mean (SD), kg/m^2	20.8 (4.8)	20.8 (4.5)	23.0 (4.6)
Race/ethnicity, *n* (%)			
Asian	59 (11.7)	86 (16.9)	59 (11.5)
Black	30 (5.9)	23 (4.5)	33 (6.4)
Hispanic American	85 (16.8)	49 (9.6)	58 (11.3)
Native American	0 (0)	5 (1.0)	0 (0)
White	321 (63.4)	341 (66.9)	354 (69.0)
Other	11 (2.2)	6 (1.2)	9 (1.8)
Geographic region, *n* (%)			
United States and Canada	168 (33.2)	222 (43.5)	163 (31.8)
Latin America	154 (30.4)	80 (15.7)	85 (16.6)
Asia-Pacific	95 (18.8)	147 (28.8)	112 (21.8)
Europe	89 (17.6)	61 (12.0)	153 (29.8)

vaccine HPV types, were detected in 3.8% (19 of 506), 1.4% (7 of 508), and 13.7% (70 of 511) of girls, boys, and women, respectively. Participants in the 16- to 23-year-old age group underwent HPV DNA testing at day 1. Among these participants, 9.6% were positive for at least 1 vaccine HPV type, indicating the possibility of ongoing HPV infection. Overall, 19.4% (97 of 501) of participants in the 16- to 23-year-old age group were positive for HPV 6, 11, 16, or 18 by serology or PCR detection of HPV DNA at day 1. Day 1 anti-HPV GMTs in 16- to 23-year-olds who were serology positive on day 1 and PCR negative through month 7 were 54.8 mMU/mL, 52.5 mMU/mL, 61.2 mMU/mL, and 51.5 mMU/mL for HPVs 6, 11, 16, and 18, respectively.

Anti-HPV Neutralizing Antibody Responses

Table 2 displays the results of the statistical analyses of noninferiority of month 7 anti-HPV GMTs comparing 10- to 15-year-old girls and boys separately with 16- to 23-year-old females for each vaccine HPV type in the PPI population. Table 2 displays the estimated GMTs (adjusted for region) for each group, along with the estimated fold difference in GMTs (younger group divided by older group) and the corresponding 95% CIs. Because the lower bound of the 95% CI for the fold difference in GMTs exceeded 0.5 for all HPV types, the statistical criterion for noninferiority for this end point was met, supporting the conclusion that anti-HPV GMTs in both 10- to 15-year-old girls and boys are noninferior to those in 16- to 23-year-old females. The observed postdose 3 anti-HPV GMTs in girls and boys were observationally higher than those that were observed in women for all 4 types. The 16- to 23-year-old females had consistently lower GMTs than the 10- to 15-year-olds at each time point for all geographic regions. The GMT fold difference between 10- to 15-year-olds and 16- to 23-year-old females in Europe was the smallest. More than 99% of participants seroconverted by month 7 to all 4 HPV types (Table 3), meeting the criterion for noninferior immune responses between populations for this primary end point as well.

Robust anti-HPV GMTs were observed by 1 month postdose 2 in all 3 groups. The GMTs at 1 month postdose 2 (month 3) for antibodies to HPVs 6, 11, 16, and 18 in girls (636 mMU/mL, 776 mMU/mL, 2834 mMU/mL, and 369 mMU/mL) and boys (678 mMU/mL, 796 mMU/mL, 3026 mMU/mL, and 414 mMU/mL) were observationally higher than those that were observed in 16- to 23-year-old females (438 mMU/mL, 550 mMU/mL, 1698 mMU/mL, and 215 mMU/mL). This is consistent with the higher immunogenicity response that was observed in girls and boys than in women at month 7. Seroconversion rates to the 4 HPV types by 1 month

TABLE 2 **Noninferiority of GMTs in Girls and Boys Versus Women at Month 7 in the PPI Population**

Assay (cLIA)	Girls		Boys		Women		GMT Ratio (95% CI)	
	n	GMT[a] (mMU/mL)	*n*	GMT[a] (mMU/mL)	*n*	GMT[a] (mMU/mL)	Girls/Women	Boys/Women
Anti–HPV 6	423	959	428	1042	320	575	1.67[b] (1.46–1.91)	1.81[b] (1.58–2.08)
Anti–HPV 11	423	1220	428	1318	320	706	1.73[b] (1.50–2.00)	1.87[b] (1.60–2.17)
Anti–HPV 16	424	4697	427	5638	306	2548	1.84[b] (1.54–2.20)	2.21[b] (1.84–2.66)
Anti–HPV 18	426	916	429	1212	340	453	2.02[b] (1.71–2.39)	2.68[b] (2.24–3.19)

[a] Based on a statistical model adjusting for region.
[b] Noninferiority $P < .001$.

TABLE 3 **Noninferiority of Seroconversion Response at Month 7 in the PPI Population**

Assay (cLIA)	Girls		Boys		Women		Difference[b] Girls-Women (95% CI)	Difference[b] Boys-Women (95% CI)
	n	Seropositive, %[a]	*n*	Seropositive, %[a]	*n*	Seropositive, %[a]		
Anti–HPV 6	423	100	428	100	320	100	0.0 (−0.9 to 1.3)[c]	0.0 (−1.0 to 1.3)[c]
Anti–HPV 11	423	100	428	100	320	100	0.0 (−0.9 to 1.3)[c]	0.0 (−1.0 to 1.3)[c]
Anti–HPV 16	424	100	427	100	306	100	0.0 (−0.9 to 1.3)[c]	0.0 (−1.0 to 1.4)[c]
Anti–HPV 18	426	100	429	99.7	340	99.1	0.8 (−0.2 to 2.5)[c]	0.6 (−0.6 to 2.4)[c]

[a] Based on a statistical model adjusting for region. Seropositivity determined relative to thresholds of 20, 16, 20, and 24 mMU/mL for HPV 6, 11, 16, and 18, respectively.
[b] Percentage point difference.
[c] Noninferiority $P < .001$.

postdose 2 exceeded 97.5% in all groups for all HPV types.

Analyses of type-specific immune responses in all type-specific HPV-naïve (ie, seronegative at day 1 and PCR-negative from day 1 through month 7) participants with serology data available from the study supported the conclusions of the per-protocol analyses. A posthoc pooled analysis that included participants from all groups who were HPV seropositive on day 1 revealed month 3 anti-HPV GMTs of 1071 mMU/mL, 4435 mMU/mL, 3955 mMU/mL, and 892 mMU/mL and month 7 anti-HPV GMTs of 1138 mMU/mL, 3909 mMU/mL, 5256 mMU/mL, and 1613 mMU/mL for HPVs 6, 11, 16, and 18, respectively. These GMTs were numerically higher than those in the PPI population, suggesting a type-specific anamnestic response in participants who had previous seroconversion as a result of infection, similar to previous findings with a monovalent HPV 16 L1 VLP vaccine.[8]

Safety and Reactogenicity

The 3-dose regimen of the vaccine generally was well tolerated in each participant group (Table 4). Discontinuations as a result of AEs were rare (3 total), and the incidence of serious AEs that occurred within 14 days of any injection was low. The most commonly reported systemic AEs were headaches (23.2%) and fever (13.1%). The proportions of participants with any vaccine-related injection site or systemic AE across the 3 groups were slightly higher after vaccination visit 1 than after vaccination visit 2 or 3.

The proportions of participants who reported at least 1 injection-site or systemic AE were lower among girls and boys than among 16- to 23-year-old females. The distributions of intensity ratings and sizes of specific injection-site AEs after each vaccination visit were comparable to those that were observed overall (ie, after any vaccination visit).

There was 1 death in the study. A 15-year-old boy died suddenly 27 days after receiving his second dose of the vaccine. The likely cause of death was a fatal ventricular arrhythmia. His autopsy was negative for any clinical findings. The investigator deemed the death unrelated to the study vaccine because of the lack of any plausible or temporal relationship. Three participants experienced nonfatal serious AEs from which they all recovered. A 15-year-old girl experienced an intentional overdose of chlorpheniramine tablets and arsenicum homeopathic tablets 13 days after receiving dose 2 that was judged by the reporting physician to not be vaccine related. A 15-year-old boy experienced lower abdominal pain accompanied by vomiting and diarrhea 9 days after receiving dose 1 that was not vaccine related, as determined by the reporting physician.

The third serious AE was vaginal hemorrhage, which occurred 26 days postdose 2 in a 13-year-old girl and was judged by the reporting physician probably to be vaccine related. After a second episode of vaginal bleed-

TABLE 4 **Clinical AEs Reported During Days 1 to 15 Postdose 1, 2, and 3 and Across All Vaccination Visits in All Participants Who Received at Least 1 Injection of Vaccine**

	Postdose 1						Postdose 2						Postdose 3						Across All Vaccinations					
	Girls		Boys		Women		Girls		Boys		Women		Girls		Boys		Women		Girls		Boys		Women	
	n	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]
Participants with follow-up	501		500		497		498		495		482		490		486		464		501		500		497	
Participants with																								
Vaccine-related AEs	330	65.9	310	62.0	355	71.4	314	63.1	232	46.9	321	66.6	308	62.9	218	44.9	310	66.8	423	84.4	396	79.2	444	89.3
Vaccine-related injection-site AEs	312	62.3	276	55.2	335	67.4	292	58.6	222	44.8	303	62.9	292	59.6	198	40.7	305	65.7	405	80.8	370	74.0	435	87.5
Vaccine-related systemic AEs	88	17.6	78	15.6	104	20.9	60	12.0	38	7.7	61	12.7	55	11.2	50	10.3	48	10.3	154	30.7	136	27.2	160	32.2
Serious AEs[b]	0	0.0	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	1	0.2	0	0.0

[a] Percentages calculated based on the number of participants with follow-up.
[b] There was 1 death and 1 nonfatal serious AE outside the 15-day postvaccination period, which are described in more detail in the text.

ing experienced by this participant 42 days after receiving dose 3 of the vaccine, the participant improved and bleeding stopped 1 day after initiation of a 7-day course of treatment with estradiol valerate tablets, medroxyprogesterone acetate tablets, and ferric hydroxide polymaltose complex tablets. Then 125 days postdose 3, vaginal bleeding was experienced again for 9 days. On consultation with 2 gynecologists, this AE was considered to be attributable to a previous condition and the last bleeding episode attributable to withdrawal of the hormonal medications listed.

The majority of injection-site AEs were mild to moderate in intensity (Table 5). Across the 3 groups, the proportion of participants who reported injection-site pain was highest after the first dose, and the proportion of participants who reported swelling and redness was higher after the second and third doses. A statistically significant higher proportion of the 16- to 23-year-olds reported injection-site erythema or pain compared with the other 2 groups (Table 5). A significantly higher proportion of 10- to 15-year-old girls and boys experienced a fever (≥37.8°C) within 5 days of any injection compared with the older population (12.8%, 13.8%, and 7.3% respectively; Table 6). Most (96.4%) of the fevers experienced were <39°C. However, 5 participants (1 girl, 3 boys, and 1 woman) experienced a maximum oral temperature of between 39.9°C and 40.9°C, and 1 girl experienced a fever of ≥40.9°C (postdose 1). A higher percentage (25.4%) of 16- to 23-year-old participants reported taking medications such as antirheumatic and anti-inflammatory drugs (eg, nonsteroidal anti-inflammatory drugs) within 15 days of each injection com-

TABLE 5 Summary of Injection-Site AEs Reported During Days 1 to 5 Postdose 1, 2, and 3 and Across All Vaccinations, in All Participants Who Received at Least 1 Injection of Vaccine

Injection Site AE	Girls (10–15 y old), *n* (%)[a]	Boys (10–15 y old), *n* (%)[a]	Women (16–23 y old), *n* (%)[a]
Pain/tenderness/soreness			
No. of participants who reported			
Postdose 1	300 (59.9)	265 (53.0)	321 (64.6)
Postdose 2	276 (55.4)	211 (42.6)	296 (61.4)
Postdose 3	288 (58.8)	185 (38.1)	295 (63.6)
Across all vaccinations	398 (79.4)	357 (71.4)	429 (86.3)
P	.004[b]	<.001[b]	
Total no. of times this AE was reported	896	686	953
Intensity of AEs[c]			
Mild	712 (79.5)	576 (84.0)	736 (77.2)
Moderate	166 (18.5)	104 (15.2)	206 (21.6)
Severe	17 (1.9)	6 (0.9)	10 (1.0)
Redness (erythema)			
No. of participants who reported			
Postdose 1	34 (6.8)	31 (6.2)	48 (9.7)
Postdose 2	52 (10.4)	35 (7.1)	50 (10.4)
Postdose 3	63 (12.9)	50 (10.3)	69 (14.9)
Across all vaccinations	101 (20.2)	93 (18.6)	130 (26.2)
P	.025[b]	.004[b]	
Total No. of times this AE was reported	149	118	170
Intensity of AEs[c]			
Mild	133 (89.3)	101 (85.6)	143 (84.1)
Moderate	14 (9.4)	11 (9.3)	16 (9.4)
Severe	2 (1.3)	4 (3.4)	5 (2.9)
Injection-site swelling			
No. of participants who reported			
Postdose 1	39 (7.8)	27 (5.4)	48 (9.7)
Postdose 2	69 (13.9)	50 (10.1)	63 (13.1)
Postdose 3	73 (14.9)	59 (12.1)	66 (14.2)
Across all vaccinations	127 (25.3)	107 (21.4)	125 (25.2)
P	.943[b]	.161[b]	
Total No. of times this AE was reported	188	138	190
Intensity of AEs[c]			
Mild	136 (72.3)	103 (74.6)	151 (79.5)
Moderate	43 (22.9)	23 (16.7)	24 (12.6)
Severe	9 (4.8)	11 (8.0)	9 (4.7)

[a] Percentages were calculated on the basis of the number of participants with follow-up (see Table 4 for denominators).

[b] *P* value for comparison with the proportion of 16- to 23-year-old females with the specific injection-site AE, unadjusted for multiple comparisons.

[c] Percentages were calculated as 100 × (No. of AEs of specific intensity/total AEs reported). Not all column percentages add up to 100% because the intensity ratings for some AEs were unknown.

TABLE 6 **Maximum Reported Temperatures During Days 1 to 5 Postdose 1, 2, and 3 and Across All Vaccinations in All Participants Who Received at Least 1 Injection of Vaccine and Provided Safety Follow-up Data**

	Postdose 1						Postdose 2						Postdose 3						Across All Vaccinations					
	Girls		Boys		Women		Girls		Boys		Women		Girls		Boys		Women		Girls		Boys		Women	
	n	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]
Participants with follow-up	495		495		489		488		486		470		485		475		448		499		500		493	
Participants with fever (temperature ≥37.8°C)	24	4.8	34	6.9	11	2.2	24	4.9	21	4.3	14	3.0	25	5.2	26	5.5	14	3.1	64	12.8	69	13.8	36	7.3[b]
Maximum temperature (oral)																								
<37.8°C (100°F)	471	95.2	461	93.1	478	97.8	464	95.1	465	95.7	456	97.0	460	94.8	449	94.5	434	96.9	435	87.2	431	86.2	457	92.7
≥37.8°C and <38.9°C (102°F)	20	4.0	26	5.3	10	2.0	21	4.3	16	3.3	11	2.3	21	4.3	21	4.4	14	3.1	53	10.6	52	10.4	32	6.5
≥38.9°C and <39.9°C (103.8°F)	3	0.6	7	1.4	1	0.2	2	0.4	4	0.8	2	0.4	4	0.8	4	0.8	0	0.0	9	1.8	14	2.8	3	0.6
≥39.9°C and <40.9°C (105.6°F)	0	0.0	1	0.2	0	0.0	1	0.2	1	0.2	1	0.2	0	0.0	1	0.2	0	0.0	1	0.2	3	0.6	1	0.2
≥40.9°C	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0

[a] Percentages were calculated on the basis of the number of participants with follow-up. Participants with multiple reported temperatures in a given range were counted only once within that range.
[b] Significantly more boys ($P < .001$) and girls ($P = .004$) experienced elevated temperatures ≥37.8°C (100°F) compared with women.

pared with boys (15.4%) and girls (18.6%). Whether this may have contributed to the differences in the reported incidence of fever between age groups is unclear.

A total of 11 participants in the 16- to 23-year-old group became pregnant during the course of the study. The outcomes of 8 pregnancies were known: 6 resulted in a live birth of a normal infant, and 2 resulted in fetal loss (1 spontaneous abortion and 1 elective abortion).

DISCUSSION

This is the first clinical study to evaluate the immunogenicity of a quadrivalent HPV vaccine in 10- to 15-year-old girls and boys. The quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP vaccine that was tested in this study is designed specifically to prevent HPV infection and related anogenital lesions, some of which may lead to precancerous and cancerous anogenital lesions later in life. One month after a 3-dose vaccination regimen, the type-specific immune responses to the vaccine that were measured in this study were numerically greater in both girls and boys than in 16- to 23-year-old females. Seroconversion for each of the 4 vaccine HPV types was achieved in >99% of participants in the study. Previous, placebo-controlled studies demonstrated that the 4 type-specific virus-neutralizing antibody responses that are induced by this quadrivalent vaccine are highly protective against persistent infection and the development of HPV-related genital lesions in HPV-naïve women.[9] The results from our study support the bridging of the HPV vaccine efficacy data that were generated in 16- to 23-year-old females who had immune responses to the vaccine, which were similar to those that we observed in 10- to 15-year-old girls.[9]

HPV infects both female and male individuals. The long-term clinical sequelae, including mortality, from HPV infection is much more common in women than it is in men.[3] However, HPV infection in men does cause significant morbidity in the form of genital warts and anogenital cancer.[3,13] Because men also are vectors for transmission of HPV infection to women, the suggestion of gender-neutral vaccination has been raised.[21] Several lines of evidence suggest that this may be an appropriate strategy. For example, previous public health experience has shown that gender-restricted vaccination programs for other viral diseases are substantially less effective than universal vaccination.[22] In addition, estimates that were obtained using mathematical modeling of the population impact of a prophylactic HPV vaccine indicate that a female-only vaccination policy likely could be 35% to 40% less efficient than vaccination of both genders.[23] The robust immune response in 10- to 15-year-old boys in our study supports the concept of gender-neutral vaccination. Studies to evaluate the efficacy of this quadrivalent HPV vaccine for prevention of infection, genital warts, and anal precancers in men are ongoing.

Administration of this quadrivalent HPV vaccine generally was well tolerated in each age group, consistent with previous findings from studies in 16- to 23-year-old females.[9] Discontinuations and serious AEs were rare. Although significantly more girls and boys experienced fevers than 16- to 23-year-old females, most (96.4%) fevers were low grade and resolved rapidly. Whether the more frequent use of antirheumatic and anti-inflammatory agents (eg, nonsteroidal anti-inflammatory drugs) by women in the study may have contributed to the difference in the incidence of fevers between age groups is unclear. In a previous study of a monovalent HPV 16 VLP L1 vaccine using the same adjuvant system in 16- to 23-year-old females, the incidence of fever was significantly higher in the vaccine group (3.5%) than in the adjuvant-only group (1.9%), with the highest percentage of subjects experiencing fever in the highest dosage (80 μg) group.[8] The incidence of fever that was reported in this study seems to be slightly higher than that reported for a recombinant hepatitis B vaccine using a similar aluminum-containing adjuvant system.[24] The incidence of fever after vaccination in 16- to 23-year-old females in our study was lower than reported (16.6%) in

15- to 25-year-olds within 7 days after administration of a bivalent HPV L1 VLP vaccine (types 16 and 18) using a different aluminum-containing adjuvant (AS04).[25]

The choice of the optimal age range for implementation of immunization programs for a new vaccine should be based on the natural history of the pathogen against which the vaccine is targeted. The peak incidence of HPV infection occurs soon after sexual debut, and risk for acquisition is strongly associated with the lifetime number of sexual partners.[3,26] In the United States, ~47% of high school students have engaged in sexual intercourse. Of these, 38% and 7.4% have reported having sex before the age of 15 and 13, respectively.[27] Early preneoplastic disease that is caused by oncogenic HPV types such as 16 and 18 often presents as low-grade dysplasia. A study that compared Papanicolaou smear diagnoses from >10 000 10- to 19-year-old subjects from health clinics in northern New England with diagnoses from Papanicolaou smears in older age groups showed that 10- to 19-year-olds have a higher percentage of SIL diagnoses and infectious processes than any older decade grouping.[11] In our study, almost 1 in 5 of the 16- to 23-year-old participants were HPV positive by serology or PCR at day 1, reflective of sexual activity in this population. This suggests that effective public health programs to prevent HPV infection and related diseases and ultimately cervical cancer through prophylactic immunization ideally should target 10- to 15-year-old girls and boys. Acceptance of such a vaccine undoubtedly will require intensive education efforts on the part of public health authorities, physicians, and other health care providers to inform parents and patients of the benefit versus risk of HPV vaccination.[28,29] Combined with cervical cancer screening programs, effective vaccination against HPV would be expected to prevent infection, reduce virus transmission, and lead to additional significant reductions in cervical cancer incidence rates and mortality.[30–32] A similar approach to reduce hepatocellular carcinoma incidence rates through universal hepatitis B vaccination has been highly successful.[33] School-based immunization requirements have had a major impact on driving the high rates of hepatitis B immunization in adolescents in the United States.[34,35] Similar approaches may be highly effective for immunization of girls and boys against HPV infection.

With any new vaccine, the duration of protection that is afforded by immunization represents a legitimate public health question. This issue is particularly relevant for HPV vaccines, because women (and men) remain at risk for HPV-related disease throughout their lives.[36] In an earlier study, type-specific antibody responses to this quadrivalent vaccine in 16- to 23-year-old females were shown to persist through 3.5 years after vaccination.[37] Although an immune correlate of protective efficacy has not been defined, the high postdose 3 anti-HPV responses in girls and boys are highly suggestive that long-term protection after vaccination is likely. The robust immune responses in 16- to 23-year-olds who were serologic or PCR positive at baseline for 1 or more of the vaccine's targeted HPV types is consistent with the anamnestic response to the HPV L1 antigen that was observed in a previous immunogenicity study.[8] This finding suggests that, if needed, booster vaccinations with this vaccine later in life would be highly effective.

CONCLUSION

Immunization of 10- to 15-year-old girls and boys with this quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP vaccine resulted in robust anti-HPV type-specific virus-neutralizing antibody responses. These neutralizing antibody responses were statistically noninferior and observationally higher (1.7- to 2.7-fold) than those observed in a population of 16- to 23-year-old females, a group in which the vaccine's 100% efficacy for the prevention of HPV-related disease was demonstrated previously.[9] Furthermore, these results support the bridging of efficacy data in 16- to 23-year-old females from large-scale studies which currently are under way in 10- to 15-year-old girls. Our findings in boys lend support for the implementation of gender-neutral immunization using this vaccine for the purpose of preventing the widespread morbidity and mortality from anogenital cancer, as well as dysplastic cervical and external genital lesions, in the general population. Long-term follow-up studies of the quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP vaccine in young adolescents are ongoing.

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