MAJOR ARTICLE

Safety and Immunogenicity of Human Papillomavirus-16/18 AS04-Adjuvanted Vaccine: A Randomized Trial in 10–25-Year-Old HIV-Seronegative African Girls and Young Women

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Background. Cervical cancer is a major public health problem for women in sub-Saharan Africa. Availability of a human papillomavirus (HPV) vaccine could have an important public health impact.

Methods. In this phase IIIb, double-blind, randomized, placebo-controlled, multicenter trial (NCT00481767), healthy African girls and young women seronegative for human immunodeficiency virus (HIV) were stratified by age (10–14 or 15–25 years) and randomized (2:1) to receive either HPV-16/18 AS04-adjuvanted vaccine (n = 450) or placebo (n = 226) at 0, 1, and 6 months. The primary objective was to evaluate HPV-16/18 antibody responses at month 7. Seropositivity rates and corresponding geometric mean titers (GMTs) were measured by enzyme-linked immunosorbent assay.

Results. In the according-to-protocol analysis at month 7, 100% of initially seronegative participants in the vaccine group were seropositive for both anti–HPV-16 and anti–HPV-18 antibodies (n = 130 and n = 128 for 10–14-year-olds, respectively; n = 190 and n = 212 for 15–25-year-olds). GMTs for HPV-16 and HPV-18 were higher in 10–14-year-olds (18 423 [95% confidence interval, 16 185–20 970] and 6487 [5590–7529] enzyme-linked immunosorbent assay units (EU)/mL, respectively) than in 15–25-year-olds (10 683 [9567–11 930] and 3743 [3400–4120] EU/mL, respectively). Seropositivity was maintained at month 12. No participant withdrew owing to adverse events. No vaccine-related serious adverse events were reported.

Conclusions. The HPV-16/18 AS04-adjuvanted vaccine was highly immunogenic and had a clinically acceptable safety profile when administered to healthy HIV-seronegative African girls and young women.

Keywords. human papillomavirus vaccine; randomized controlled trial; female adolescents; women; immunogenicity; safety; Africa.

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Persistent infection with oncogenic human papillomavirus (HPV) is established as the necessary cause of cervical cancer [1, 2]. Sub-Saharan Africa has the highest age-standardized incidence and mortality rates for cervical cancer in the world [3]. Women frequently present with advanced disease due to lack of effective cervical cancer screening programs, and treatment facilities are limited [4–7]. Therefore, HPV prophylactic vaccination would be of particular value in sub-Saharan African countries.

GlaxoSmithKline Biologicals SA has developed a prophylactic HPV vaccine based on L1 proteins of HPV-16 and HPV-18, formulated with the AS04-adjuvant system [8]. In clinical trials, the vaccine was immunogenic and had a clinically acceptable safety profile in girls and young women aged ≥9 years [9–11] and was highly efficacious in girls and young women aged 15–25 years from diverse ethnic and geographic origins [11].

There are currently no data on the immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine in sub-Saharan Africa. Therefore, a randomized placebo-controlled trial was conducted in Dakar, Senegal, and in Mwanza, Tanzania, to evaluate immunogenicity and safety of the vaccine in healthy human immunodeficiency virus (HIV)–negative girls and young women aged 10–25 years.

METHODOLOGY

Trial Design

This was a phase IIIb, randomized, double-blind, placebo-controlled trial (NCT00481767) with 2 parallel groups conducted at 2 centers in sub-Saharan Africa (Service des Maladies Infectieuses, CHU Fann, Dakar, Senegal and the Mwanza Intervention Trials Unit of the National Institute for Medical Research, Tanzania) from October 2007 through July 2010. Potential participants from schools, a teacher training college and family planning clinics were invited to attend for screening. Approximately half of the participants in Senegal were recruited from an urban setting (a city high school) and half from a rural setting (a fishing village); all participants in Tanzania were recruited from an urban setting (Mwanza city). Those eligible for enrollment included healthy HIV-seronegative girls and young women 10–25 years old at first vaccination, who had a negative urine pregnancy test and ≤6 lifetime sexual partners. Exclusion criteria are shown in the supplementary material.

Eligible participants were randomized (2:1) to receive 3 doses of HPV-16/18 AS04-adjuvanted vaccine or aluminum hydroxide ($Al(OH)_3$) (placebo) at 0, 1, and 6 months. Enrollment was stratified by age (10–14 and 15–25 years), with approximately one-third of participants enrolled in the 10–14-year age stratum, and balanced between centers. The randomization list was computer generated at GlaxoSmithKline Biologicals SA, and subjects were assigned to groups according to an Internet-based randomization blocking scheme. Visits were scheduled for screening (within 30 days before administration of the first vaccine or placebo dose), day 0, and months 1, 2, 4, 6, 7, 10, and 12. Investigators, study staff, and participants in each country were blinded to vaccine assignment until all subjects in that country had completed the month 12 visit. Participants in the placebo group were given the opportunity to be vaccinated with *Cervarix*® (GlaxoSmithKline Vaccines) and participants in the vaccine group were offered an alternative licensed vaccine (hepatitis B vaccine in Tanzania and *Mencevax*® [GlaxoSmithKline Vaccines] in Senegal).

The primary objective was to evaluate antibody responses against HPV-16 and HPV-18 1 month after the last vaccine dose (month 7) in girls and young women aged 10–14 years and 15–25 years. Secondary objectives were to evaluate antibody responses at months 2 and 12 and to evaluate safety and reactogenicity throughout the trial.

The trial protocol and other relevant documents were approved by the national ethics committee of Senegal (Comité National des Etudes en Recherche sur la Santé) and the Western Institutional Review Board, Olympia, Washington, for the Senegalese center and the ethics committees of the National Institute for Medical Research, Tanzania, and the London School of Hygiene and Tropical Medicine, London, for the Tanzanian center. The trial was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). An Independent Data Monitoring Committee met quarterly to review safety results and make recommendations concerning continuation.

Before the performance of any trial-specific procedures, signed assent or thumb-printed assent (for illiterate participants) was obtained from participants below the legal age of consent (<18 years old), and written informed consent was obtained from their parents or legally acceptable representative. Participants above the legal age of consent provided written or thumb-printed informed consent themselves.

Vaccine and Placebo

Each dose of HPV-16/18 AS04-adjuvanted vaccine *Cervarix*® (GlaxoSmithKline Vaccines) contained 20 μg each of HPV-16 and HPV-18 L1 viruslike particles, 50 μg of 3-O-desacyl-4′-monophosphoryl lipid A, and 500 μg of $Al(OH)_3$. The placebo contained 500 μg of $Al(OH)_3$ only. Vaccine and placebo were supplied as a liquid (0.5 mL) in individual prefilled syringes to be administered intramuscularly into the deltoid muscle of the nondominant arm.

Serological Evaluation of HPV-16 and HPV-18 Antibodies

Blood samples were collected at day 0 (before vaccination) and at months 2, 7, and 12 for the measurement of HPV-16 and HPV-18 antibodies by enzyme-linked immunosorbent assay (ELISA) at a central laboratory (GlaxoSmithKline Biologicals SA), as described elsewhere [12]. Seropositivity was defined as an antibody titer greater than or equal to the assay threshold of 8 ELISA units (EU)/mL for HPV-16 and 7 EU/mL for HPV-18 [12].

Safety Evaluation

Solicited local symptoms (pain or swelling at injection site) and general symptoms (arthralgia, fatigue, fever, gastrointestinal symptoms, headache, myalgia, rash, or urticaria) occurring

within 7 days after each vaccination and unsolicited adverse events (AEs) occurring within 30 days after each vaccination were recorded by a trained field worker, using a diary card. Investigators documented the presence or absence of urticaria or rash within 30 minutes after vaccination. Grade 3 symptoms were defined as swelling at the injection site >50 mm in diameter, fever >39°C (axillary), urticaria distributed on ≥4 body areas, and, for other symptoms, as preventing normal daily activity.

Serious AEs (SAEs), other medically significant conditions (ie, AEs prompting emergency room or physician visits that were not related to common diseases), potential new onset of chronic diseases including new onset of autoimmune diseases (eg, autoimmune disorders, asthma, type 1 diabetes, allergies) [10], and pregnancies were reported from month 0 to month 12. No further vaccine or placebo doses were given to participants who became pregnant and any pregnancies were to be followed to completion.

Blood samples were collected at screening and at months 7 and 12 for routine hematological and biochemical parameters and HIV testing. Urine samples were collected for pregnancy testing on site before each vaccination.

Statistical Considerations

Safety analyses were based on the total vaccinated cohort, which included all participants who received at ≥1 vaccine or placebo dose. Immunogenicity analyses were based on the according-to-protocol (ATP) cohort for immunogenicity, which included evaluable participants meeting all eligibility criteria, complying with the procedures and intervals defined in the protocol (including receipt of the scheduled number of doses), with no elimination criteria during the trial, for whom immunogenicity data were available. Two ATP immunogenicity cohorts were defined: a month 7 cohort, used for analyses at month 2 and 7, and a month 12 cohort, used for analyses at month 12. Analyses of immune responses were stratified by serostatus for the corresponding antigen at baseline.

The target enrollment was approximately 666 subjects (444 for the vaccine group and 222 for the placebo group), with equal numbers enrolled at the 2 centers. It was assumed that 30% of participants would drop out, be initially seropositive for HPV-16/18, or meet elimination criteria excluding them from the ATP immunogenicity cohort. With this sample size, the power to demonstrate a lower limit of the 95% confidence interval (CI) >90% if the true seroconversion rate was 98%, was 94% for participants aged 10–14 years and 99% for participants aged 15–25 years.

Within each vaccine group, for each age stratum, seroconversion and seropositivity rates are reported for anti–HPV-16 and anti–HPV-18 with exact 95% CIs, and geometric mean titers (GMTs) with 95% CIs. GMTs were calculated by taking the anti-log of the mean of the log titer values. Antibody titers below the cutoff of the assay were given an arbitrary value of half the cutoff value.

The percentage of doses followed by solicited AEs and unsolicited AEs, and the percentage of participants with ≥1 event for other safety outcomes, are reported with exact 95% CIs. Descriptive statistics for hematology and biochemistry were calculated, as were the percentages of participants with values outside the normal ranges at each time point. The study was not designed or powered to compare differences in GMTs between the 2 age strata, nor to compare differences between vaccine and placebo groups for safety outcomes, and statistical testing was not done.

RESULTS

Trial Population

Overall, 676 participants were enrolled and vaccinated (342 in Senegal and 334 in Tanzania) and 623 (92%) completed the trial up to month 12 (Figure 1). The most common reason for discontinuation was withdrawal of consent (4%). No participant was withdrawn because of an AE or SAE. The ATP immunogenicity cohort included 561 (83%) participants for analysis at month 7 and 547 (81%) participants for analysis at month 12. The most common reason for elimination from ATP immunogenicity cohorts was missing essential serological data (56 participants [8%]) (Figure 1).

All participants were of African origin and were HIV seronegative at baseline. Demographic characteristics were generally similar between the vaccine and placebo groups (Table 1) and between participants from Senegal and Tanzania (data not shown). Overall, the mean age was 16.9 years, the mean body mass index was 19.3 kg/m^2, and 97.3% of participants were nonsmokers. The majority of participants (79.2%) in the ATP cohort for immunogenicity for analysis at month 7 were seronegative at baseline for both anti–HPV-16 and anti–HPV-18 antibodies (Table 2).

The majority of subjects in both groups received all 3 vaccine doses (411 participants [91.3%] in the vaccine group and 200 participants [88.5%] in the placebo group).

Immunogenicity

At month 2, all initially seronegative participants in the vaccine group (in the ATP cohort for immunogenicity) had seroconverted for anti–HPV-16 and anti–HPV-18 antibodies and remained seropositive up to month 7 (320 of 320 participants [100%; 95% CI, 98.9%–100%] for anti–HPV-16; 340 of 340 [100%; 95% CI, 98.9%–100%] for anti–HPV-18). At month 12, all initially seronegative participants in the vaccine group remained seropositive for anti–HPV-16 (312 of 312 participants; 100% [95% CI, 98.8%–100%]), and all except 1 (a 13-year-old girl from Tanzania) remained seropositive for anti–HPV-18 (330 of 331; 99.7% [98.3%–100%]).

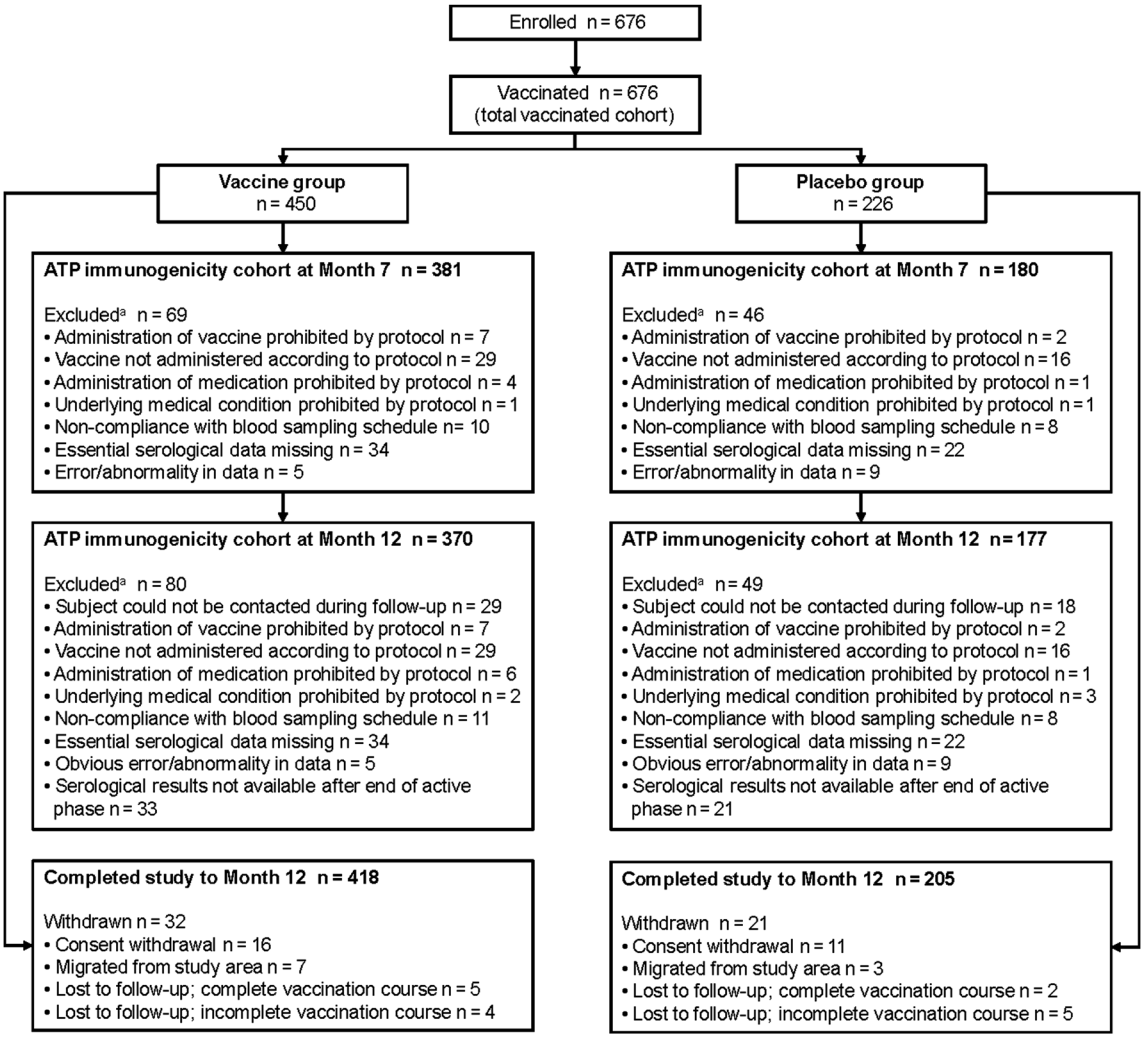

Figure 1. Flow of participants through the trial. Excluded participants may have had more than one reason for elimination from the according-to-protocol (ATP) immunogenicity cohort; therefore, the sum of reasons for elimination is more than the total number of participants excluded.

In the vaccine group, high anti–HPV-16 and anti–HPV-18 antibody titers were observed at month 2 in initially seronegative participants in both age strata, and an increase in GMTs was observed from month 2 to month 7 (Table 3 and Figure 2). Anti–HPV-16 and anti–HPV-18 titers at month 7 were approximately 1.7-fold higher in 10–14-year-olds (18 423 [95% CI, 16 185–20 970] and 6487 [5590–7529) EU/mL, respectively) than in 15–25-year-olds (10683 [9567–11 930] and 3743 [3400–4120] EU/mL, respectively) (Figure 3). After the peak antibody response observed at month 7 in the vaccine group, a decline in antibody titers was observed at month 12 (Table 3 and Figure 2). GMTs at each time point were similar for participants from Senegal and those from Tanzania (data not shown).

All participants in the vaccine group who were seropositive for HPV-16 or HPV-18 at baseline remained seropositive at months 2, 7, and 12. After vaccination, GMTs for these initially seropositive participants were similar to those observed after vaccination in initially seronegative participants (Table 3). In the placebo group, no effect on HPV titers was seen after placebo administration, irrespective of initial HPV serostatus (Table 3).

Safety

During the 7-day postvaccination period, the incidence of any solicited symptom was higher for vaccine recipients than for placebo recipients (after 72.0% [95% CI, 69.5%–74.5%] and 60.7% [56.8%–64.5%] of doses, respectively), mainly owing to a higher incidence of local symptoms (59.7% [95% CI, 57.0%–62.4%] and 43.1% [39.2%–47.0%], respectively). General symptoms were reported after 35.1% of doses in both groups. The incidence of any symptom (local or general) was highest after the first dose and decreased with subsequent doses.

Table 1. Demographic and Baseline Characteristics for the Total Vaccinated Cohort

Characteristic	Vaccine (n = 450)	Placebo (n = 226)	Total (N = 676)
Age, mean ± SD, y	16.9 ± 4.36	16.8 ± 4.16	16.9 ± 4.29
Age group, No. (%)			
10–14 y	155 (34.4)	77 (34.1)	232 (34.3)
15–25 y	295 (65.6)	149 (65.9)	444 (65.7)
BMI, mean ± SD, kg/m^2	19.4 ± 3.91	19.3 ± 3.67	19.3 ± 3.83
Smoking status, No. (%)			
Smoker	0 (0.0)	3 (1.3)	3 (0.4)
Former smoker	12 (2.7)	3 (1.3)	15 (2.2)
Nonsmoker	438 (97.3)	220 (97.3)	658 (97.3)
Menarchal status, No. (%)			
Postmenarchal	321 (71.3)	162 (71.7)	483 (71.4)
Premenarchal	129 (28.7)	63 (27.9)	192 (28.4)
Missing	0	1	1

Abbreviations: BMI, body mass index; SD, standard deviation.

Injection site pain was the most frequently solicited local symptom in both groups (Figure 4). Although pain was reported more often for vaccine recipients (55.9%) than for placebo recipients (38.7%), only 2 doses (0.2%) in the vaccine group were followed by grade 3 pain. Pain was reported less frequently in both vaccine and placebo groups in Senegal (39.5% and 22.2%, respectively) than in Tanzania (73.2% and 55.5%, respectively). No urticaria or rash within 30 minutes of vaccination was reported. The most frequently solicited general symptoms in both groups were headache and fever (Figure 4). No grade 3 solicited general symptoms were reported.

Table 2. Serological Status at Baseline for the According-to-Protocol Immunogenicity Cohort.

	Participants, No. (%)		
Serological Status by Age Stratum	Vaccine (n = 381)	Placebo (n = 180)	Total (n = 561)
Age 10–14 y			
HPV-16 seronegative	130/142 (91.5)	59/65 (90.8)	189/207 (91.3)
HPV-18 seronegative	128/141 (90.8)	56/66 (84.8)	184/207 (88.9)
Both seronegative[a]	120/140 (85.7)	53/65 (81.5)	173/205 (84.4)
Age 15–25 y			
HPV-16 seronegative	190/237 (80.2)	97/113 (85.8)	287/350 (82.0)
HPV-18 seronegative	212/235 (90.2)	98/114 (86.0)	310/349 (88.8)
Both seronegative[a]	175/234 (74.8)	89/113 (78.8)	264/347 (76.1)
All participants			
HPV-16 seronegative	320/379 (84.4)	156/178 (87.6)	476/557 (85.5)
HPV-18 seronegative	340/376 (90.4)	154/180 (85.6)	494/556 (88.8)
Both seronegative[a]	295/374 (78.9)	142/178 (79.8)	437/552 (79.2)

Data for serological status at baseline are summarized for the month 7 according-to-protocol cohort for immunogenicity. Data represent No. of participants with specified serostatus/No. of participants with serological data for specified antibody (%).

Abbreviation: HPV, human papillomavirus.

[a] Both HPV-16 and HPV-18 seronegative.

During the 30-day postvaccination period, unsolicited symptoms were reported after 25.3% and 30.2% of doses in the vaccine and placebo groups, respectively (Table 4). The most frequently reported unsolicited symptoms were related to infections (after 11.1% and 14.0% of doses in vaccine and placebo groups, respectively), with malaria reported after 5.1% and 6.7% of doses, respectively. The incidence of suspected or proven malaria was higher in both the vaccine and placebo groups in Tanzania (21.7% and 29.2%, respectively) than in Senegal (3.5% and 2.7%, respectively).

No deaths were reported up to month 12 (Table 4). Nonfatal SAEs (mainly related to malaria) were reported by 31 participants; none of which were considered by the investigators to be related to vaccination. The proportions of participants with medically significant conditions, potential new onset chronic diseases, or autoimmune diseases were low and similar in both groups (Table 4).

Fifteen pregnancies were reported to month 7, and 9 were reported between months 7 and 12. Overall, 14 participants gave birth to live infants (4 were premature), 6 underwent elective termination, 2 experienced spontaneous abortion, 1 had an ectopic pregnancy and 1 was lost to follow up with unknown pregnancy outcome. No apparent congenital anomalies were reported for the live births. Two premature infants died (1 in each group); in both cases the investigator determined that there was no reasonable possibility that the prematurity was caused by vaccination. There were no clinically

Table 3. GMTs for Anti–HPV-16 and Anti–HPV-18 Antibodies by Baseline Serostatus, Vaccine Group, and Age Stratum (According-to-Protocol Immunogenicity Cohort)

Vaccine Group and Age Stratum	Month	Seronegative at Baseline				Seropositive at Baseline			
		GMT,[a] EU/mL				GMT,[a] EU/mL			
		No.	Value	LL	UL	No.	Value	LL	UL
Anti–HPV-16									
Vaccine									
10–14 y	0	130	4.0	4.0	4.0	12	20.3	12.4	33.1
	2	130	5279.8	4742.7	5877.8	12	6039.7	4220.2	8643.5
	7	130	18 422.8	16 184.8	20 970.4	12	20 518.0	12 821.7	32 834.1
	12	128	4010.2	3276.0	4909.1	11	4086.4	2409.2	6931.0
15–25 y	0	190	4.0	4.0	4.0	47	27.1	20.0	36.6
	2	190	3525.9	3239.3	3837.8	47	4699.7	3723.3	5932.2
	7	190	10 683.0	9566.7	11 929.5	47	10 587.7	8490.7	13 202.6
	12	184	2357.1	2073.2	2679.9	45	2955.0	2273.6	3840.5
All ages	0	320	4.0	4.0	4.0	59	25.5	19.8	33.0
	2	320	4154.3	3874.5	4454.4	59	4945.7	4062.6	6020.9
	7	320	133 30.2	12 199.5	14 565.7	59	12112.7	9858.8	14 881.9
	12	312	2931.3	2611.9	3289.8	56	3149.3	2501.9	3964.1
Placebo									
10–14 y	0	59	4.0	4.0	4.0	6	15.8	8.6	29.1
	2	59	4.2	3.8	4.5	5	10.7	3.3	34.5
	7	59	4.4	4.0	4.9	6	10.3	3.3	32.8
	12	59	4.2	3.9	4.5	6	13.8	6.4	30.1
15–25 y	0	97	4.0	4.0	4.0	16	20.5	12.9	32.6
	2	96	4.2	4.0	4.4	16	12.4	6.6	23.0
	7	97	4.4	4.0	4.8	15	18.4	10.6	31.9
	12	94	4.6	4.1	5.1	15	19.8	9.2	42.7
All ages	0	156	4.0	4.0	4.0	22	19.1	13.4	27.2
	2	155	4.2	4.0	4.3	21	11.9	7.3	19.7
	7	156	4.4	4.1	4.7	21	15.6	9.7	24.9
	12	153	4.4	4.1	4.8	21	17.9	10.2	31.3
Anti–HPV-18									
Vaccine									
10–14 y	0	128	3.5	3.5	3.5	13	12.0	10.0	14.4
	2	128	3018.9	2662.0	3423.7	13	2994.6	1942.9	4615.6
	7	128	6487.3	5589.7	7529.1	13	5694.4	3372.7	9614.6
	12	126	1403.5	1140.7	1726.8	12	1633.3	866.5	3078.7
15–25 y	0	212	3.5	3.5	3.5	23	14.0	10.6	18.6
	2	212	2009.4	1824.3	2213.2	23	2337.7	1738.8	3142.7
	7	212	3742.7	3400.2	4119.8	23	2925.6	2442.9	3503.6
	12	205	855.5	762.0	960.5	22	859.7	633.2	1167.3
All ages	0	340	3.5	3.5	3.5	36	13.3	11.0	16.0
	2	340	2342.2	2164.0	2535.0	36	2556.4	2019.9	3235.4
	7	340	4603.8	4222.8	5019.2	36	3721.0	2949.1	4694.9
	12	331	1032.9	926.1	1152.0	34	1078.3	800.0	1453.2
Placebo									
10–14 y	0	56	3.5	3.5	3.5	10	9.3	7.5	11.5
	2	55	3.6	3.4	3.7	10	7.0	4.9	10.0
	7	56	3.6	3.5	3.8	10	5.8	3.8	8.7
	12	56	3.5	3.5	3.5	10	4.8	3.3	6.9
15–25 y	0	98	3.5	3.5	3.5	16	17.7	9.5	33.0
	2	98	3.8	3.5	4.2	14	17.5	9.0	33.9

Table 3 continued.

Vaccine Group and Age Stratum	Month	Seronegative at Baseline				Seropositive at Baseline			
		GMT,[a] EU/mL				GMT,[a] EU/mL			
		No.	Value	LL	UL	No.	Value	LL	UL
	7	98	3.9	3.5	4.2	16	13.9	6.9	28.0
	12	96	3.9	3.5	4.2	15	14.8	7.1	30.8
All ages	0	154	3.5	3.5	3.5	26	13.8	9.3	20.5
	2	153	3.7	3.5	3.9	24	11.9	7.7	18.4
	7	154	3.8	3.5	4.0	26	9.9	6.2	15.9
	12	152	3.7	3.5	4.0	25	9.4	5.7	15.4

Abbreviations: EU, enzyme-linked immunosorbent assay units; GMT, geometric mean antibody titer; HPV, human papillomavirus; LL, lower limit of 95% confidence interval; UL, upper limit of 95% confidence interval.

The according-to-protocol (ATP) immunogenicity cohort at month 7 was used for the months 0, 2, and 7 time points; the ATP immunogenicity cohort at month 12, for the month 12 time point.

[a] Antibody titers below the cutoff of the assay were given an arbitrary value of half the cutoff for the purpose of GMT calculation (ie, 4.0 EU/mL for anti–HPV-16 and 3.5 EU/mL for anti–HPV-18).

significant hematological or biochemistry abnormalities, and no relevant differences in laboratory parameters from baseline to month 7 or month 12 between the vaccine or placebo groups in either age stratum (data not shown). The Independent Data Monitoring Committee supervising this trial stated that there were no serious safety concerns.

DISCUSSION

This is the first evaluation of the HPV-16/18 vaccine in healthy HIV-seronegative adolescent girls and young women in sub-Saharan Africa. To include participants with different genetic, environmental, ethnic, or cultural backgrounds, we conducted the trial in 2 geographic locations, Senegal in West Africa and Tanzania in East Africa.

The HPV-16/18 vaccine was found to be highly immunogenic for both antigens in girls or young women 10–14 or 15–25 years old. All initially seronegative participants seroconverted at month 2 for both anti–HPV-16 and anti–HPV-18 antibodies and remained seropositive up to month 7. The persistence of antibodies in this trial followed the expected pattern; that is, after a peak response 1 month after completion of the 3-dose vaccination course, a decline in antibody titers was observed at month 12. After vaccination, antibody titers for initially seropositive participants were similar to those observed after vaccination in initially seronegative participants.

As observed elsewhere, anti–HPV-16 and anti–HPV-18 antibody titers at month 7 were higher in participants aged 10–14 years than in those aged 15–25 years [13]. The GMTs for the 2 age strata at month 7 in the current trial were in the same range as those observed in participants of a similar age who received similar vaccine lots in a previous immunogenicity and safety trial conducted in Europe (Denmark, Estonia, Finland, Greece, The Netherlands, and Russia) (Figure 3) [13]. This was reassuring, because a number of potential factors could have affected immunogenicity in African populations, including differences in HLA types and the prevalence of immunomodulating diseases, such as malaria and helminth infections, which are not common in Europe. GMTs 1 month after the last vaccine dose in a subset of subjects in the vaccine group (n = 56) who had malaria (presumptive or proven) reported as an AE within the 30-day postvaccination period (anti–HPV-16, 10 213 [95% CI, 7017–14 865] EU/mL; anti–HPV-18, 3878 [2783–5402] EU/mL) were in line with those observed in the overall ATP immunogenicity cohort. It is also encouraging that GMTs were similar in the 2 participating African countries, despite a difference in malaria epidemiology between these countries [14].

In previous clinical trials, high vaccine-induced GMTs in previously uninfected women have been linked with protection against persistent HPV-16/18 infection and related disease [11, 15–17]. By analogy, therefore, the immune responses observed in our trial would be expected to confer similar protection (although not measured). GMTs at months 7 and 12 in our trial were well above those previously observed in women who have cleared a natural infection (Figure 2) [16]. Furthermore, peak GMTs were also higher than those observed in a previous study in women aged 15–25 years vaccinated with the HPV-16/18 vaccine, in whom sustained efficacy was observed against persistent infection and high-grade cervical lesions associated with HPV-16/18 (Figure 2) [18]. Long-term immunogenicity was not evaluated in the current study, but in previous trials antibody titers elicited by the HPV-16/18 vaccine were sustained at levels several-fold above

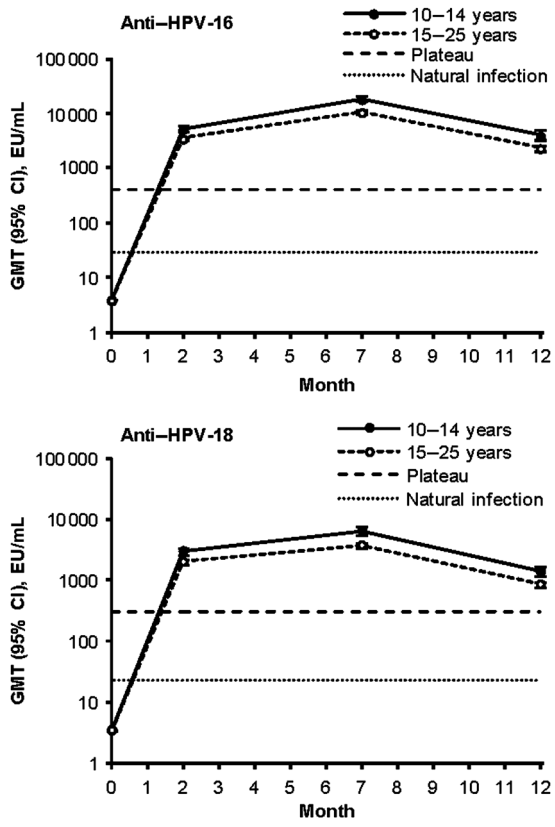

Figure 2. Kinetics of anti–human papillomavirus (HPV) 16 and anti–HPV-18 antibody responses in initially seronegative participants in the vaccine group of the according-to-protocol (ATP) immunogenicity month 7 cohort. The ATP immunogenicity cohort at month 7 was used for the months 0, 2, and 7 time points; the ATP immunogenicity cohort at month 12, for the month 12 time point. Natural infection indicates GMT in women who had cleared a natural infection [16]; plateau, GMT at plateau level (months 45–50) from a previous study in women aged 15–25 years, in which sustained protection with the HPV-16/18 AS04-adjuvanted vaccine was shown up to 6.4 years after first vaccination [18]. Abbreviations: CI, confidence interval; EU, enzyme-linked immunosorbent assay units.

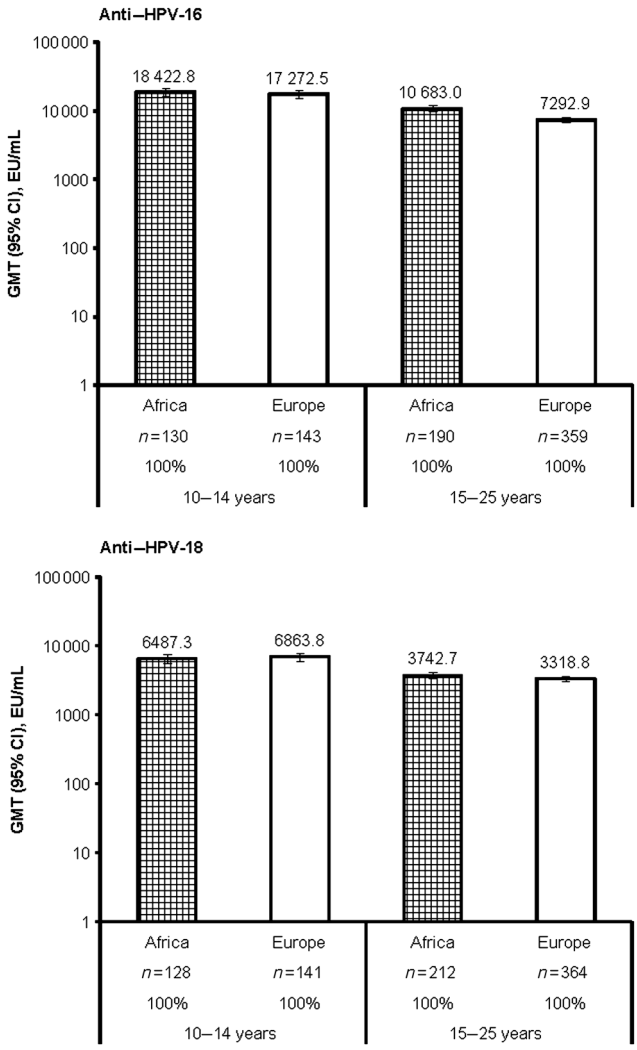

Figure 3. Geometric mean titers (GMTs) for anti–human papillomavirus (HPV)-16 and anti–HPV-18 at month 7 in initially seronegative participants in the vaccine group of the according-to-protocol ATP immunogenicity month 7 cohort. Values above bars show GMTs by stratum; percentages below bars, seroconversion rates; n, number of evaluable participants at month 7. Abbreviations: CI, confidence interval; EU, enzyme-linked immunosorbent assay units. Data for Europe are from Pedersen et al [13].

those observed for natural infection, for up to 8.4 years after vaccination [19].

The higher immunogenicity of a 3-dose schedule of the HPV-16/18 vaccine in girls aged 10–14 years compared with young women aged 15–25 years, observed in this and other clinical trials, has prompted discussion on whether an alternative 2-dose schedule would be suitable for vaccination of preteens/adolescents, and studies are ongoing to evaluate this. If a 2-dose schedule is found to provide acceptable immunogenicity and efficacy [20], it could potentially reduce healthcare costs and logistical barriers and may improve vaccination coverage and patient compliance. This would be particularly relevant in regions such as sub-Saharan Africa.

The HPV-16/18 vaccine had a clinically acceptable safety profile when administered to healthy, HIV-seronegative, African girls and young women aged 10–25 years. Overall, the reactogenicity and safety profiles were similar in both groups, except for injection site pain, which was more frequently reported in vaccine recipients; however, only 2 (first) doses of vaccine were followed by grade 3 injection site pain. The reactogenicity profile of the HPV-16/18 vaccine in this trial differed in some respects from that previously reported in a pooled safety analysis of data from almost 30 000 adolescent girls and women aged ≥10 years from diverse ethnic and geographic origins outside of Africa [10]. The reported incidence of solicited local and general symptoms was generally lower in

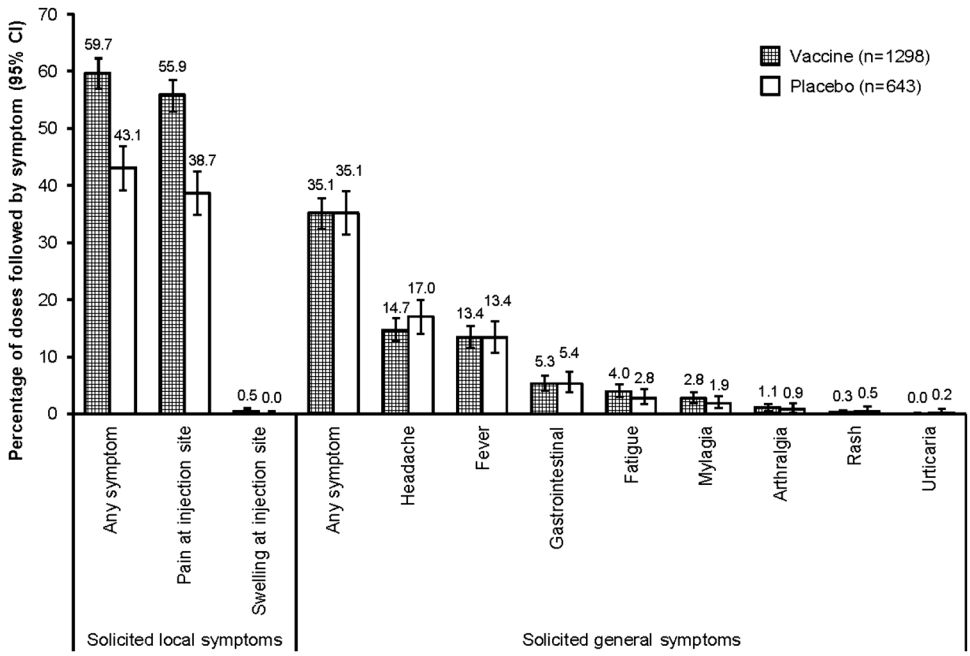

Figure 4. Incidence of solicited symptoms during the 7-day period after any dose (total vaccinated cohort). Abbreviations: CI, exact 95% confidence interval; n, total number of documented doses.

this African trial than in the pooled safety analysis. For example, the incidence of pain or swelling after any vaccine dose was 56% and 0.5%, respectively, in this trial, compared with approximately 75% and 25%, respectively, in the pooled analysis. However, fever was reported at a higher incidence in the current trial (13%) than in the pooled safety analysis (7%). The reported incidence of SAEs or other medically significant conditions was also generally higher in this trial (4% and 69%, respectively) than in the pooled safety analysis (approximately 3% and 20%, respectively). This increased incidence was mainly due to malaria and other events related to infections. Variability in AE reporting across countries could be due to a number of factors, such as cultural differences in reporting of AEs and geographic differences in the prevalence or incidence of infectious and other diseases. The instrument used to record AEs could also positively or negatively affect reporting rates: a trained field worker was used to collect AE data in the current study, whereas self-reporting was used in most other trials.

In this study, we selected a population that was at low risk for HPV infection and consequently for other sexually transmitted infections, which may have affected vaccine responses. Furthermore, the trial enrolled healthy participants and consequently vaccine responses were not evaluated in girls and young women with characteristics that may be of relevance in an African setting, such as malnutrition, HIV, other sexually transmitted infections, or other clinically significant medical conditions. A supplementary study is being conducted to examine the effect of helminth and malaria infection on vaccine immunogenicity in Tanzania. Of importance for African settings, trials will also evaluate the immunogenicity and safety of the HPV-16/18 vaccine in HIV-infected women (NCT00586339 and NCT01031069).

Initial HPV-16/18 seropositivity rates in the current trial were lower than those previously reported for women without cervical cancer in Africa [21, 22]. This lower HPV baseline seroprevalence may be explained by the younger age of participants (including preteens and adolescents) and their lower risk of HPV infection; by design, they had to have a low number of lifetime sexual partners and all were HIV seronegative. In contrast, the populations in previous epidemiological studies were more heterogeneous and included women at high risk for HPV infection.

Data from this trial support the use of the HPV-16/18 vaccine in healthy, HIV-seronegative girls and young women in sub-Saharan Africa. Effective cervical cancer screening coverage in sub-Saharan Africa is generally low, particularly in rural areas, and women frequently present with advanced disease and treatment facilities are limited [3–6]. Therefore, HPV-16/18 prophylactic vaccination before the onset of sexual debut offers the potential to decrease cervical cancers incidence and related mortality. The challenge for the future will be to scale up effective national HPV vaccination programs in the region.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of

Table 4. Summary of Safety and Reactogenicity (Total Vaccinated Cohort)

	Vaccine		Placebo	
	No.[a]	% (95% CI)	No.[a]	% (95% CI)
Unsolicited symptoms in 30-d period after any vaccine dose				
No. of documented doses	1298		643	
Any symptom	329	25.3 (23.0–27.8)	194	30.2 (26.6–33.9)
Grade 3 symptom	6	0.5 (.2–1.0)	1	0.2 (.0–.9)
Causal relationship to vaccination[b]	3	0.2 (.0–.7)	4	0.6 (.2–1.6)
≥1% incidence (in either group) after any dose				
Malaria	66	5.1 (4.0–6.4)	43	6.7 (4.9–8.9)
Headache	41	3.2 (2.3–4.3)	19	3.0 (1.8–4.6)
Dysmenorrhea	24	1.8 (1.2–2.7)	10	1.6 (.7–2.8)
Abdominal pain	17	1.3 (.8–2.1)	7	1.1 (.4–2.2)
Vertigo	15	1.2 (.6–1.9)	4	0.6 (.2–1.6)
Cough	13	1.0 (.5–1.7)	8	1.2 (.5–2.4)
Nasopharyngitis	10	0.8 (.4–1.4)	12	1.9 (1.0–3.2)
Other safety events from month 0 to month 12				
No. of participants	450		226	
Death	0	0.0 (.0–.8)	0	0.0 (.0–1.6)
Serious adverse event	17	3.8 (2.2–6.0)	14	6.2 (3.4–10.2)
Adverse event leading to premature discontinuation	0	0.0 (.0–.8)	0	0.0 (.0–1.6)
Medically significant condition	312	69.3 (64.8–73.6)	170	75.2 (69.1–80.7)
New onset chronic disease	11	2.4 (1.2–4.3)	11	4.9 (2.5–8.5)
New onset autoimmune disease	2	0.4 (.1–1.6)	2	0.9 (.1–3.2)

Abbreviations: CI, exact confidence interval.

[a] Number of doses followed by symptom (for unsolicited symptoms) or number of participants with event (for other safety end points).

[b] As judged by the investigator.

data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. D. W. J., J. C., J. B., B. K., and A. A. have received grant funding from GlaxoSmithKline (GSK) Biologicals SA; P. M. has received grants from GSK Research & Development; M. L., M. H. (consultant from the contract research organization Chiltern), F. T., and D. D. are currently employed by the GlaxoSmithKline group of companies; F. T. and D. D. also have stock ownership in the GlaxoSmithKline group of companies. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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