Immunogenicity of bivalent HPV vaccine among partially vaccinated young adolescent girls in Uganda

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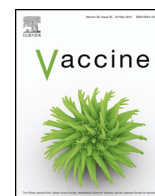**26** PUBLICATIONS **274** CITATIONS

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Immunogenicity of bivalent HPV vaccine among partially vaccinated young adolescent girls in Uganda

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ABSTRACT

Background: Investigations of vaccine efficacy and immunogenicity for adult females receiving fewer than three doses of human papillomavirus (HPV) vaccine have suggested protection against infection and precancerous lesions. We investigated the immunogenicity of bivalent HPV vaccines among adolescent girls from Uganda who received one, two, or three vaccine doses.

Methods: Young girls vaccinated through a government program in Uganda were invited to participate. HPV16- and HPV18-specific antibodies were measured at $\geq$24 months after the last vaccine dose using an enzyme linked immunoassay in girls who received one (n = 36), two (n = 145), or three (n = 195) doses.

Results: Nearly all subjects (99%) were HPV16 and HPV18 seropositive at the time of blood-draw. Geometric mean antibody levels (GMTs) were: $\text{HPV16}_{1\text{-dose}}$ = 230 EU/mL, $\text{HPV16}_{2\text{-dose}}$ = 808 EU/mL, and $\text{HPV16}_{3\text{-dose}}$ = 1607 EU/mL; $\text{HPV18}_{1\text{-dose}}$ = 87 EU/mL, $\text{HPV18}_{2\text{-dose}}$ = 270 EU/mL, and $\text{HPV18}_{3\text{-dose}}$ = 296 EU/mL. The GMT ratio for 2:3 doses was 0.50 (HPV16) and 0.68 (HPV18) and did not meet the non-inferiority criteria (i.e., lower bound of 97.5% confidence interval of the GMT ratio greater than 0.50). The GMT ratio for 1:3 doses for HPV16 and HPV18 was inferior, but absolute GMTs for one dose were higher than adult women who received one dose (HPV16 = 124 EU/mL, HPV18 = 69 EU/mL) where efficacy has been demonstrated.

Conclusions: Even though immunogenicity with less than three doses did not meet a priori non-inferiority thresholds, antibody levels measured $\geq$24 months after last dose were similar to those of adult women who have been followed for more than eight years for efficacy.

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1. Introduction

Cervical cancer is a major global public health problem with more than 530,000 new cases and over 275,000 deaths annually and disproportionately affects developing countries [1]. High income countries have reduced mortality through well-organized, systematic screening programs that detect precancerous lesions [2,3]. The investment and expense of establishing comprehensive cervical cancer screening programs based on European models are too great for low-resource countries [4,5]; however, new approaches, such as visual inspection with acetic acid (VIA) followed by immediate and appropriate treatment, show some promise in making screening feasible, affordable, and cost-effective [6].

The past decade has brought significant change to the landscape of cervical cancer prevention with the development and availability of two highly efficacious vaccines (bivalent Cervarix®, developed by GlaxoSmithKline, Belgium; quadrivalent Gardasil®, developed by Merck & Co., USA). The bivalent vaccine includes HPV serotypes 16 and 18, and the quadrivalent vaccine includes HPV 6, 11, 16 and 18 serotypes. These vaccines prevent nearly 100% of precancerous lesions caused by two carcinogenic types of human papillomavirus (HPV), HPV16 and 18, associated with 70% of all cervical cancers

[7–9]. Efficacy of both HPV vaccines has been established in adult female populations [7,8] and immuno-bridging studies among girls demonstrated non-inferiority of vaccine-induced immunity [9–16]. The results of these immuno-bridging studies have indicated that antibody levels generated among girls aged 9–15 years were several folds higher than adult women [9–13,16]. In addition, among all vaccine recipients antibodies peak soon after the administration of the third dose and wane for a period of months before plateauing around 24 months [7,10–12,14–16]. Recent studies have found that two doses of bivalent HPV vaccine given to adolescents produced a similar antibody response [14,16] compared to adult women (among whom efficacy has been demonstrated) [7,8] that was maintained at 24, 36, and 48 months after the first dose [14,16,18]. Findings from natural history studies have suggested that modest levels of antibodies resulted in partial protection from future infections [19] and these levels were significantly lower than antibody levels among adolescents girls who received three or fewer doses of the bivalent or quadrivalent HPV vaccine [18], even when measured eight years after vaccination [7,10,11]. This suggests that protection may not require all three vaccine doses. Previous investigations of vaccine efficacy [20] and immunogenicity [21] in adult women receiving less than three doses suggest that two and even one dose may be sufficient for protection.

Country experience to-date has demonstrated feasible models of HPV vaccine delivery that have achieved high coverage in a variety of low-, middle-, and high-income countries [22–26]. However, completion of all three doses can be challenging [22–25,27–29], with variable three-dose completion rates of 18–33% in the US [22], 48–85% in Canada [22], 61–90% in Uganda [23], 68–88% in India [23], 83–99% in Vietnam [23], and 44–77% in Australia [26], depending upon year of measurement, age, delivery strategy, and/or geographic location. The feasibility of delivering HPV vaccine is challenged by needing to administer three doses within six months [23,27–29], mobilizing health workers for delivery [24], coordinating with schools for school-based immunizations [22–24,27–30], and financing the costs of delivery [24,31]. If fewer than three doses of HPV vaccine afforded protection, identified barriers to three-dose vaccine implementation, including costs of vaccine procurement and delivery, could be reduced. In the absence of efficacy trials, information on the immunogenicity of HPV vaccine among girls who receive one or two doses would address a programmatic need for countries where delivery could be easier, resulting in higher completion rates and reduced overall costs.

This study compared HPV16 and HPV18 antibody responses elicited by the bivalent vaccine among young adolescent girls in Uganda who received one or two doses relative to girls who received all three doses at ≥24 months after last dose.

2. Materials and methods

2.1. Study design and population

We designed a cross-sectional follow-up study of the immunogenicity of bivalent HPV vaccine in adolescent girls who were vaccinated as part of a government-run HPV vaccination demonstration program in Uganda implemented from October 2008 to October 2009 in Nakasongola District [23]. Nakasongola District is a rural pastoralist community in central Uganda with a population of 200,000, largely ethnically and economically homogenous. All girls in the district aged 10 years were eligible for HPV vaccination, administered by immunization program vaccinators at schools or community-outreach venues on a 0-, 1-, and 6-month schedule [23]. Three-dose completion among girls aged 10 years was 52% in the first year and 60% in the second year of implementation, with notable dropout rates [23].

Our primary objective was to compare the immune response more than 24 months after last dose of vaccine received among three groups—receipt of one, two, or three doses of vaccine. Girls who received one or two doses were compared to those who received three doses with non-inferiority defined similarly to other studies [14,18,32,33]. Recruitment to each study group targeted up to 200 girls to address our objective.

All girls listed on HPV vaccine registers from the district's nine sub-counties who received at least one dose of vaccine from October 1, 2008 through October 31, 2009 were eligible for the study. Recruitment for each study group started in the sub-county with the highest number of partially vaccinated girls and continued to the next sub-county until the group enrollment target of 200 was reached. The entire sub-county was notified of the study through sensitization meetings with district leaders, home visits to inform parents and the girls, and announcements and leaflets, which acted as an invitation to all parents of vaccinated girls. However, if recruitment to a group was completed from only one sub-county (as was expected for the three-dose group), then subsequent invitations for study participation were made to parents/guardians of girls from the other study groups, i.e., those who had received only one or two doses.

Interested parents attended an informational meeting for the study, where the objectives and procedures were explained, and if willing, written consent was obtained from both the parent/guardian and the participant. Eligibility included age less than 18 years, received at least one dose of bivalent vaccine during the time period of the demonstration program, in good health and afebrile, and able to comply with the study procedures during the study. Fig. 1 outlines the sampling frame. Data collected included verification of vaccine doses received, dates of vaccination, demographics, height, weight, arm circumference, age at menarche, and mosquito net use (as a proxy for possible malaria exposure). All subjects were recruited, consented and had blood drawn within one to four days in a single location before moving to the next location in the district. All subjects exited the study once the blood draw procedures were completed.

2.2. Study procedures

Consented participants provided approximately 10 mL of blood at a central location (either the local health facility or school), which was centrifuged and serum aliquotted to three vials on site, then stored in cryo-containers at $-70\,^\circ$C according to standard procedures. Samples were sent to the HPV Immunology Laboratory of the National Cancer Institute (Fredrick, Maryland, USA) and tested by enzyme-linked immunosorbent assay (ELISA) for HPV16 and HPV18 antibody concentrations. Antibody levels, expressed as ELISA units (EU)/mL, were calculated by interpolation of optical density values from the standard curve by averaging the calculated concentrations from all dilutions that fell within the working range of the standard curve. The laboratory-determined seropositivity cutoffs for HPV16 and HPV18 were 8 EU/mL and 7 EU/mL, respectively [34,35].

2.3. Data analysis

Geometric mean antibody levels (expressed as GMTs) are presented. To test non-inferiority of one and two vaccine doses relative to three doses, we calculated the ratios of type-specific GMTs (1:3 dose and 2:3 dose), with multiplicity-adjusted 97.5% confidence intervals. We defined non-inferiority as the lower bound of the confidence interval of the GMT ratio greater than 0.50 [18,32,33]. Positivity at the laboratory's suggested cutoff (defined above) was used to dichotomize results. We report GMT ratios and absolute GMTs by vaccine dose received. While thus far no immune
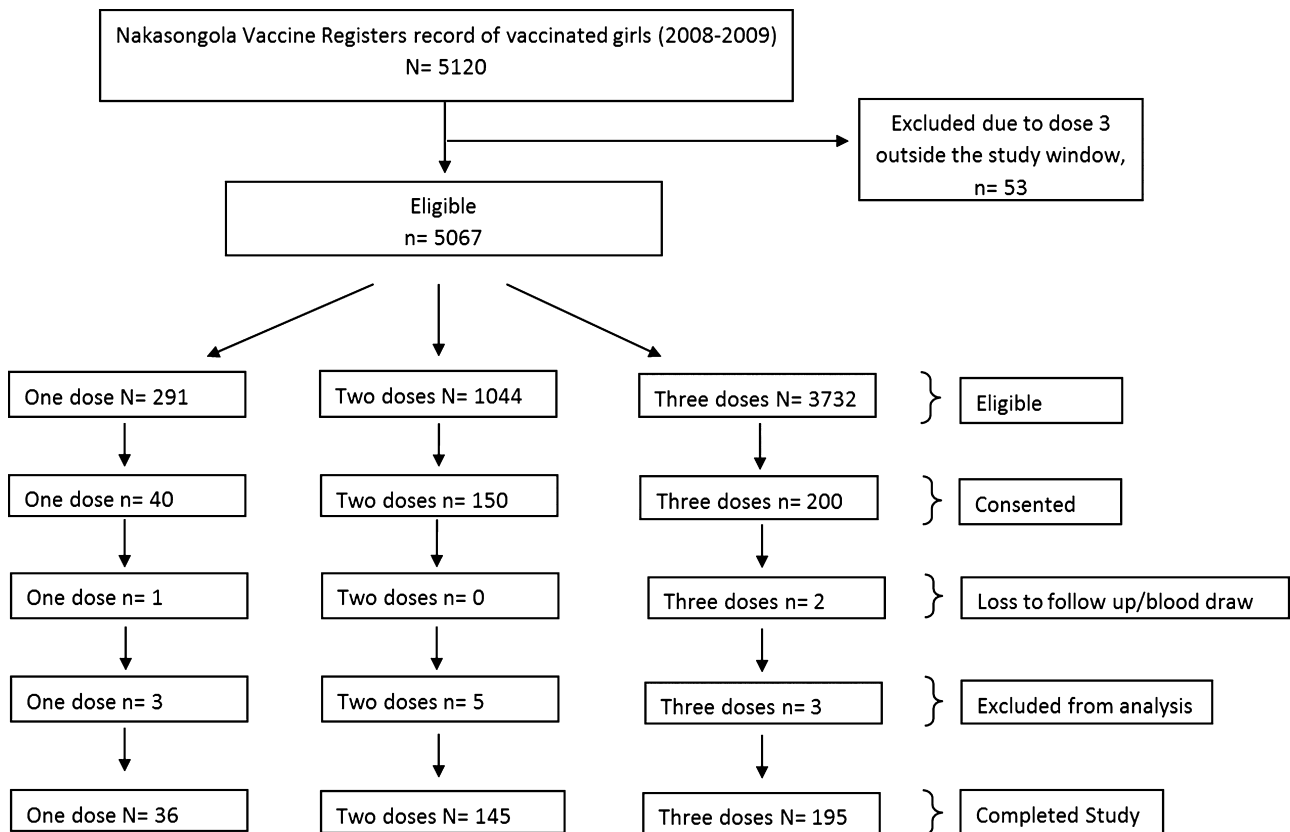
Fig. 1. Study flow, bivalent HPV vaccine immunogenicity among young adolescent girls, Uganda.

correlate of protection has been identified for HPV vaccine, we reasoned that because of the near complete vaccine efficacy observed in adult women who received all three doses [7,8], the minimum antibody levels among the three-dose group could serve as an immunogenicity benchmark. Antibody levels among girls in our study who received one or two doses of HPV vaccine were compared with the lowest antibody level measured among girls who received three doses (160 EU/mL for HPV16 and 18 EU/mL for HPV18). The percent of participants in each dosing group in our study whose titers were above these levels was calculated.

We conducted an exploratory analysis to compare GMTs for adolescent girls who received one or two doses of HPV vaccine to natural infection-induced HPV16 and HPV18 antibody levels measured among young women who participated in a bivalent HPV vaccine trial in Costa Rica (CVT), and those who received fewer than three doses in the CVT [21,34]. Briefly, in this trial women 18–25 years were randomized to receive either bivalent HPV16/18 or hepatitis A vaccine at the recommended 0, 1, 6 month schedule and were followed for four years. While women were randomized to get three vaccine doses, 20% of women in both arms received fewer than three doses, mainly due to pregnancies and colposcopic referral [21]. We compared HPV16/18 antibody responses of the Ugandan girls to post hoc analysis of sera from CVT participants who received one, two, or three vaccine doses. The same ELISA assay performed by the same laboratory was used to evaluate the antibody responses.

All statistical analyses were performed in Stata SE, version 11 (Stata Corp., College Station, TX, USA).

2.4. Ethics

This study was approved by ethics committees in the United States and Uganda: PATH Research Ethics Committee, Seattle, WA,

USA; National Cancer Institute, Special Studies Institutional Review Board, Rockville, MD, USA; the Uganda Virus Research Institute, Science and Ethics Committee, Entebbe, Uganda; and the Uganda National Council of Science and Technology, Kampala, Uganda.

3. Results

Registries for two rounds of vaccinations indicated that in Nakasongola District there were 5120 girls vaccinated from October 1, 2008 through October 31, 2009: 291 girls who had received one dose, 1044 who had received two doses, and 3785 girls had received all three doses of bivalent HPV vaccine. Fifty-three girls were excluded from study eligibility as their third dose of vaccine was received after October 31, 2009. All girls in all nine sub-counties who had received one or two doses of HPV vaccine were invited to participate, but only girls from two sub-counties who had received all three doses were invited, as the recruitment goal had been reached within the first few days of study enrollment (Fig. 1). There were 150 girls who received two doses of HPV vaccine who consented for the study of which 145 completed all study procedures and were included in our analysis. For girls who received only one dose of HPV vaccine, 40 consented, 39 completed the study procedures, and 36 were included in our analysis. We did not achieve the recruitment totals for the one-dose and two-dose groups because new information from the parents indicated that some girls identified on the vaccine registry as partially vaccinated were subsequently tracked by health workers and given missed doses. Even though the vaccination history was not updated on the vaccine register, vaccination cards in the possession of the parents clearly indicated missed doses were received and this information was taken as the "gold standard" in determining final vaccination status.

Table 1
Participant demographics.

	Girls who received 3 doses ($n = 195$)	Girls who received 2 doses ($n = 145$)	Girls who received 1 dose ($n = 36$)
Mean age at vaccination (years) (range)	10.8 (10–11)	10.6 (10–11)	11.0 (11–11)
Mean follow-up time from first dose received to blood draw (months) (range)	38 (29–43)	41 (30–49)	40 (29–48)
Mean follow-up time from last dose received to blood draw (months) (range)	38 (29–43)	39 (29–49)	33 (17–48)
Body mass index			
Mean (range)	17.8 (10.3–29.3)	18.6 (14.0–28.5)	18.1 (13.7–27.2)
Median (Interquartile range)	17.6 (16.2–19.5)	18.3 (16.8–19.8)	17.8 (16.4–20.4)
Menarche before vaccination (number of girls)	0	8 (5.5%)	0

The mean age at vaccination was 10 years for all three groups (Table 1). The mean follow-up time between first dose of vaccine and time of blood collection for antibody measurement varied by study group—38 months for the one-dose, 41 months for the two-dose, and 40 months for the three-dose group—due to recruitment from two vaccination rounds where round 1 started October 1, 2008, and round 2 began April 1, 2009. The mean follow-up time between the last dose of vaccine and time of immunogenicity measurement also varied by study group—38 months for the one-dose group, 39 months for the two-dose group, and 33 months for the three-dose group—due to the longer follow-up time for those with fewer doses and those who received vaccine during round 1 (starting October 1, 2008). Body mass index (BMI), arm circumference, menarche, and mosquito net use were similar across the study groups. Almost all participants were vaccinated before menarche (100% of the one- and three-dose and 95% of the two-dose group).

Among girls who received three doses of vaccine, 89% ($n = 159$) received their 2nd and 3rd doses within pre-specified dosing windows. Although not powered to detect differences in antibody levels between girls receiving all three doses within the dosing window and those with more extended time between doses, HPV16 antibody and HPV 18 antibody levels trended toward slightly higher values amongst the latter (data not shown). Among girls who received only two doses of vaccine, 75% ($n = 106$) received the second dose within the pre-specified dosing window; 5% received the second dose earlier; and 20% received the second dose >90 days after dose 1. No statistical differences in antibody levels for HPV16 and HPV 18 were found between these dosing groups in post hoc analysis.

Nearly all subjects (99.25%) seroconverted.[1] HPV16 and HPV18 antibody levels for each group at each study visit are shown in Table 2 and Fig. 2. Antibody GMTs were 230 EU/mL for HPV16 and 87 EU/mL for HPV18 for the one-dose group, 808 EU/mL for HPV16 and 270 EU/mL for HPV18 for the two-dose group, and 1607 EU/mL for HPV16 and 296 EU/mL for HPV18 for the three-dose group. The GMT ratio between girls who received two doses and three doses was 0.50 (HPV16) and 0.68 (HPV18) and did not meet our pre-defined criteria for non-inferiority (Table 2). The same threshold for non-inferiority between those who received one dose and those who received three was also not met. However, approximately 60% (HPV16) and 86% (HPV18) of one-dose vaccines had antibody levels above the minimum levels observed among the three-dose vaccines (Table 2). For two-dose recipients, 86% (HPV16) and 98% (HPV18) had HPV16/18 levels that were above the minimum levels observed among the three-dose vaccines. HPV16 and HPV18 antibody levels among girls who had received two doses and those who received three doses did not differ by age, length of time between
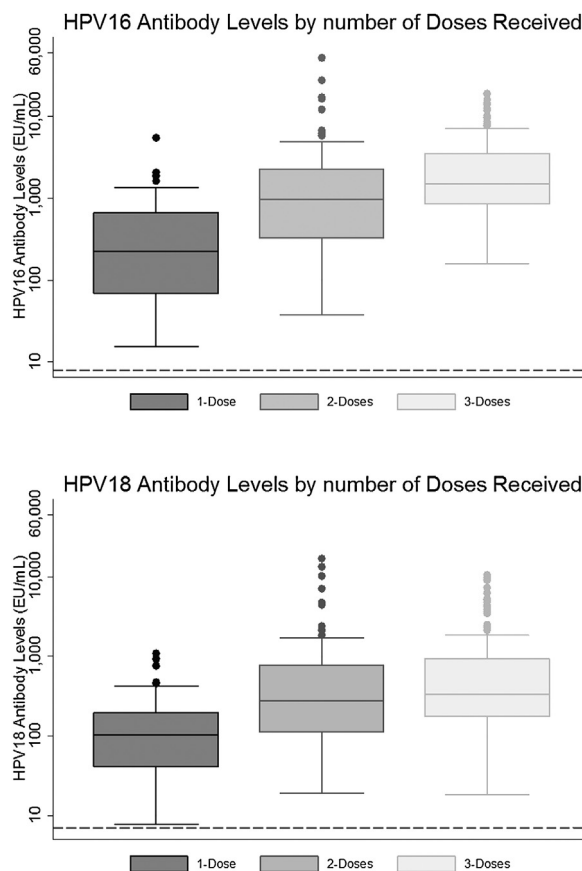

Fig. 2. HPV16 antibody titers box plot at time of immunogenicity measurement among young adolescent girls who received two or three doses of vaccine, Uganda. Levels among seropositives only. Dashed lines are assay seropositivity cutoff levels (8 EU/mL for HPV16 and 7 EU/mL for HPV18).

first dose of vaccine and immunogenicity measurement, BMI, or onset of menarche (data not shown).

HPV16 and HPV18 antibody levels for each of our study groups were slightly higher (but not statistically significantly) compared to those of adult participants in the Costa Rica HPV16/18 Vaccine Trial (CVT) with the same number of doses measured 36 months after first dose (Fig. 3), among whom vaccine efficacy for one and two doses has been demonstrated [21]. Both HPV16 and HPV18 antibody levels in our study from all groups were higher than those found in natural history studies of HPV infection that demonstrated immune protection from subsequent infection (Fig. 3) [19,21,35].

4. Discussion

We measured the immune response to bivalent HPV vaccine more than three years after vaccination among African adolescent

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[1] One participant in the three-dose group was seronegative for both HPV16 and HPV18; one individual in the two-dose group was seronegative for both HPV16 and HPV18; and two participants in the two-dose group were seronegative for either HPV16 or HPV18. One individual in the one-dose group was seronegative for HPV18.

Table 2
HPV16- and HPV18-specific antibody geometric mean titers and antibody ratios of GMTs (1:3 doses and 2:3 doses), young adolescent females, Uganda.

No. of doses	N	GMTs (95% CI)	Ratio (97.5% CI)	n (%) above lowest antibody levels of girls who received 3 doses
HPV16				
1	36	229.86 (139.27–379.38)	0.14 (0.09–0.23)	22 (61.11)
2	145	808.38 (631.86–1034.22)	0.50 (0.37–0.69)	125 (86.21)
3	195	1607.92 (1381.78–1871.07)	Referent	194 (100.00)[a]
HPV18				
1	36	86.87 (54.98–137.23)	0.22 (0.13–0.37)	31 (86.11)
2	145	270.21 (213.15–342.55)	0.68 (0.49–0.95)	143 (98.62)
3	195	395.51 (331.15–472.37)	Referent	194 (100.00)[a]

girls who received less than three doses. Even though we did not meet the pre-defined threshold for non-inferiority, our findings are similar to previous studies of immunogenicity in the context of two doses of bivalent HPV vaccine [14,16] (Table 3). We also observed that HPV16 antibody levels among African adolescent girls who received two doses of bivalent HPV vaccine were higher than those measured among adult women who received two doses of the same vaccine (808 EU/mL [632–1034] vs. 519 EU/mL [452–594]) [21] and for whom efficacy has been demonstrated [20]. While surrogate markers of immune protection or minimal levels required for protection have not been established yet, given that antibody levels among our study population were similar to those of CVT participants where vaccine efficacy was demonstrated for two- and even one-dose group, we may expect protection in this population as well.

Our finding among the three-dose recipients is also encouraging and indicates that vaccine induced immune responses among these participants is robust and comparable to other settings [14–16] (Table 3). While absolute levels may not be directly comparable due to use of different laboratories and reagents, there remained a similarity of patterns of immunogenicity. Schwarz et al. reported immunogenicity at 48 months after the first dose among adolescents who received three doses of bivalent HPV vaccine: HPV16 antibody GMTs of 2375 EU/mL, and HPV18 antibody GMTs of 797 EU/mL [15]. Sow et al. investigated the safety and immunogenicity of bivalent HPV vaccine given to 10- to 25-year-old females in Senegal and Tanzania participating in a randomized trial and found antibody concentrations at months 7 and 12 (when peak levels are expected) were higher (HPV16 GMT range: 3276–4909 EU/mL) [36] than in our study which measured levels at least 24 months after last dose, when levels have declined and are in the plateau phase [7,11,14,15,37] (Table 3).

HPV16 antibody levels at >24 months after the dose among girls receiving one dose in this study were nearly double (230 EU/mL [139–379]) than those from adult women who received one dose in the CVT (124 EU/mL [92–168]) measured at a similar time point; levels of HPV18 antibody concentrations were slightly higher, though not to the same degree (87 EU/mL [55–137] vs. 69 EU/mL [52–90]) [21]. All GMTs reported among girls who only received one dose of vaccine in our study were higher than levels shown to confer partial protection in natural history studies [19]. Safaeian and colleagues found HPV16 GMTs at 60 EU/mL and HPV18 GMTs at 28 EU/mL were partially protective against future infections in women previously infected [19]. Even though an immune correlate of protection for bivalent HPV vaccine has yet to be determined, if the antibody concentration levels measured in this study hold steady over the long term, girls who received as few as one dose of bivalent HPV vaccine in this pilot program in Uganda may still receive the protective benefits of the vaccine. Further follow-up of these girls is warranted in the coming years, especially as they are entering an age when sexual exposure to HPV will be greatest [38].

In addition to the clinical benefit that partially vaccinated adolescent girls may receive, there is a larger programmatic context to consider should fewer than three doses of HPV vaccine be considered sufficient. As noted previously, even in well-resourced national HPV vaccination programs implemented in high-income countries, such as Australia, the United Kingdom, and the United States, there are significant challenges to ensuring that girls who initiate HPV vaccination complete the three dose series [22,29]. Barriers include loss to follow-up, absenteeism in school (for programs utilizing schools as a vaccination venue), misinformation on the need for all three doses, and clinicians' reluctance to encourage vaccination [22–25,27–30]. These challenges are magnified in developing countries with fewer human resources and less
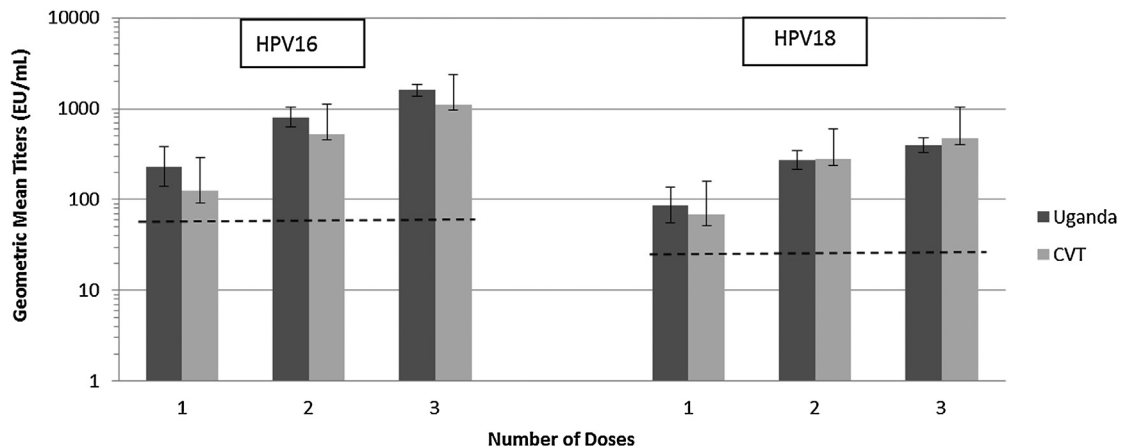

Fig. 3. HPV16 and HPV18 specific antibody geometric mean titers by number of doses received among girls aged 10–12 years in Uganda (≥24 months after last vaccine dose) and women aged 18–25 years in Costa Rica (≥24 months after first dose). Dashed lines are levels from naturally infected women in Costa Rica HPV16/18 Vaccine trial that were shown to confer immune protection from future infections (60 EU/mL for HPV16 and 28 EU/mL for HPV18) [34,35].

Table 3
Studies reporting long-term immunogenicity[a] of the HPV16/18 AS04-adjuvanted vaccine among adolescent and adult females.

Author (reference)	Ages (years)/Sex	Doses	Sched. (months)	@mo. 2	@mo. 7	@mo. 12/13	@mo. 18	@mo. 24/27	@mo 36	@mo. 48
Reported HPV16 GMTs, EU/mL (95% CI, if known), at month since first dose										
LaMontagne (this issue)	10–17 yo F	1	0, 1, 6						230 (139–379)	
		2							808 (632–1034)	
		3							1608 (1382–1871)	
Safaeian [21]	18–25 yo F	1	0, 1, 6						136	
		2							433	
		3							899	
Romanowski [14]; Schwarz [15]; Romanowski [16]	9–14 yo F	2	0, 6		11,067 (9190–13,328)			1702 (1416–2045)	1595 (1298–1960)	1320 (1084–1607)
Lazcano-Ponce [45]	9–10 yo F	3	0, 6, 60		10,442 (9894–11,020) After 2 doses			1432 (1357–1510) @mo.27 After 2 doses		
Pedersen [10]; Petaja [11]	10–14 yo F	3	0, 1, 6		17,273 (15,118–19,734)			4074 (3027–5484)	3445 (2581–4599)	2862 (2129–3847)
Rivera Medina [12]; Schwarz [15]	10–14 yo F	3	0, 1, 6		19,882			3226		2375 (2206–2557)
Kim [13]	10–14 yo F	3	0, 1, 6		19,620 (17,189–22,395)					
Schmeink [17]	9–15 yo F	3	0, 1, 6		21,713					
Sow [36]	10–14 yo F	3	0, 1, 6		18,423 (16,185–20,970)	4010 (3276–4909)				
Garcia-Sicilia [46]	10–18 yo F	3	0, 1, 6		18,965 (16,849–21,347)					
Wheeler [47]	11–18 yo F	3	0, 1, 6		Range: 21,235–22,813	Range: 8020–10,292				
Pedersen [10]; Petaja [11]	15–25 yo F	3	0, 1, 6		7293 (6624–8030)			1426 (1207–1686)	1372 (1163–1619)	1186 (1007–1397)
Romanowski [14]; Schwarz [15]; Romanowski [16]	15–25 yo F	3	0, 1, 6		10,332 (8329–12,792)			1865 (1505–2311)	1592 (1283–1976)	1420 (1134–1777)
Esposito [48]	15–25 yo F	3	0, 1, 6	3117	10,312	11,885				
		3	0, 1, 12	3195		@mo.13				
Lazcano-Ponce [45]	18–25 yo F	3	0, 1, 6		6991 (6333–7717)			1035 (953–1125) @mo.27		
Konno [37]	20–25 yo F	3	0, 1, 6		8033 (a)	2899 (a)		1522 (b)		
Reported HPV18 GMTs, EU/mL (95% CI, if known), at month since first dose										
LaMontagne (this issue)	10–17/F	1	0, 1, 6						87 (55–137)	
		2							270 (213–343)	
		3							396 (331–472)	
Safaeian [21]	18–25/F	1	0, 1, 6						69	
		2							245	
		3							389	
Romanowski [14]; Schwarz [15]; Romanowski [16]	9–14/F	2	0, 6		5510 (4646–6535)			702 (563–876)	689 (530–896)	543 (427–691)

Lazcano-Ponce [45]	9–10/F	3	0, 6, 60		5837 (5517–6175) After 2 doses		619 (583–657) @mo.27 After 2 doses		
Pedersen [10]; Petaja [11]	10–14/F	3	0, 1, 6		6864 (5976–7883)		1412 (1079–1850)	1188 (887–1591)	941 (715–1238)
Rivera Medina [12]; Schwarz [15]	10–14/F	3	0, 1, 6		8262		1263		865 (797–938)
Kim [13]	10–14/F	3	0, 1, 6		9895 (8674–11,287)				
Schmeink[17]	9–15/F	3	0, 1, 6		8839				
Sow [36]	10–14/F	3	0, 1, 6		6487 (5590–7529)	1403 (1141–1727)			
Garcia-Sicilia [46]	10–18/F	3	0, 1, 6		6902 (6061–7861)				
Wheeler [47]	11–18/F	3	0, 1, 6		Range: 7075–8316	Range: 2395–3272			
Pedersen [10]; Petaja [11]	15–25/F	3	0, 1, 6		3319 (3023–3644)		616 (515–738)	575 (481–689)	470 (395–559)
Romanowski [14]; Schwarz [15]; Romanowski [16]	15–25/F	3	0, 1, 6		4262 (3572–5084)		728 (588–900)	712 (560–906)	605 (476–768)
Esposito [48]	15–25/F	3	0, 1, 6	2271	3964	4501 @mo.13			
		3	0, 1, 12	2338					
Lazcano-Ponce [45]	18–25/F	3	0, 1, 6		3483 (3164–3834)		438 (395–485) @mo.27		
Konno [37]	20–25/F	3	0, 1, 6		4076 (a)	1352 (a)	627 (b)		

^a Absolute levels for GMTs measured in EU/mL between studies may not be directly comparable due to use of different laboratories, procedures, source of reagents, and time of specimen processing. Information provided as reference only.

program funding to track populations for subsequent doses, especially in rural areas where the majority of the population may still reside [23,24,27,28,30]. If two doses of HPV vaccine were enough then these challenges could be reduced and the financial resources required for delivery of three doses [31] could be reprogrammed to meet additional health needs. A two-dose schedule may also facilitate easier integration of HPV vaccine with other services, such as twice-yearly deworming programs, thereby further reducing health-worker burden for vaccine delivery [23]. The governments of Chile [39] and Quebec, Canada [40] have already made the decision to recommend two doses of HPV vaccine in their program, and the government of South African recently launched their nationwide HPV vaccination program for young girls using a two-dose schedule of 0, 6 months [41]. Additional results from phase 4 follow-up studies linking efficacy endpoints with receipt of fewer doses of HPV vaccine [42] will provide further evidence as to whether two doses of HPV vaccine is sufficient for long-term protection. Recent approval by the European Medicines Agency for a two-dose vaccine regimen for the both the bivalent and quadrivalent HPV vaccine for 9–14 year old girls [43] contributed to the evidence base used by the Strategic Advisory Group of Experts on Immunization (SAGE) to make a global recommendation to the World Health Organization for two-doses, provided girls start the series prior to their 15th birthday [44].

While the results of our study of immunogenicity among partially vaccinated adolescent girls in Uganda is thought-provoking and provides further empirical data to inform the debate on fewer than three-dose HPV vaccination schedules, it is important to note a few limitations. First, this was not a randomized controlled trial of the efficacy of one-dose and two-dose HPV vaccination compared to three doses. As such, inferences of efficacy for fewer than three doses cannot be made. Second, we did not achieve an optimal sample size, especially among those who received only one dose of bivalent HPV vaccine, to test with sufficient power our primary hypothesis of non-inferiority. However, despite this, GMTs among adolescents who received only one dose measured nearly three years after vaccination were still higher than women who received one dose of HPV vaccine for which no breakthrough cases have been detected four years after vaccination [20,21]. Additional larger studies of girls who were partially vaccinated are warranted to both confirm the initial findings of our study and independently validate GMT levels three years after vaccination. Third, our study was on the bivalent HPV vaccine and results may not be inferred to the quadrivalent HPV vaccine. Further, we used the same ELISA and calibrated standards to measure immunogenicity as that used in the bivalent HPV vaccine trial among adult women in the CVT [19–21,34]. Thus, in principle our results can be compared with studies using ELISAs with data reported based on GlaxoSmithKline EU/mL standards; however, inter-laboratory differences could affect results. We also note that our study was among generally healthy African adolescent girls, but we have not investigated if there are differences among those HIV-positive or otherwise immunosuppressed, as well as other potential differences between girls in this study and those who were vaccinated in the original demonstration program. Lastly, the implications of immunogenicity levels among girls who received only one dose of HPV vaccine in our study are speculative and meant to generate discussion for further studies. Caution should be taken when interpreting these results.

Adolescent girls in Uganda who received fewer than three doses of bivalent HPV vaccine in a routine vaccination pilot developed a robust immune response that may afford protection, despite presenting lower concentrations of antibodies compared to those who received all three doses. If a two-dose regimen is proven sufficient to adequately protect vaccinated girls from future infections with vaccine-type HPV, low-resource settings, like Uganda, may benefit from cost reductions and increased programmatic feasibility of vaccine delivery.

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