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Antibody responses among adolescent females receiving two or three quadrivalent human papillomavirus vaccine doses at standard and prolonged intervals

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Contributions

LW, STC, SF, CH, DB participated in study design, recruitment, acquisition of data, analysis and interpretation of findings, manuscript preparation. RH participated in analyses, interpretation of findings, and manuscript preparation. ERU, GP participated in laboratory evaluations, interpretation of findings, and manuscript preparation. LJ, AB, KK, BP, KE, MM, JS participated in acquisition of data, study recruitment, interpretation of findings. All authors participated in the development of the manuscript and approved the final version for journal submission.

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Abstract

Background—The originally recommended dosing schedule, 0, 2, 6 months, for the 3-dose quadrivalent human papillomavirus vaccine (4vHPV) was often not followed, resulting in longer than recommended intervals between doses and interest in the effect of prolonged intervals. Recent two-dose recommendations require investigations into the effect of delaying dose 2.

Methods—This multi-site, prospective study enrolled healthy 9-17 year old girls (n=1321) on the day of or within 28 days following a third dose of 4vHPV vaccination. Antibody titers to 4vHPV types were measured at one and six months post-dose 3 from all participants and post-dose 2 from participants who were on time for dose 3. To compare antibody responses, participants were categorized into groups: second and third doses on time (control group); on-time dose 2, substantially late dose 3 (group 2); substantially late dose 2, on-time dose 3 (group 3); both doses substantially late (group 4). Analyses compared age-adjusted geometric mean titers (GMTs) at one-month and six-months post-dose 3, effect of delaying the second dose, and two versus three doses as well as post-dose 2 GMTs, stratified by age.

Results—Compared to on-time dosing, one-month post-dose 3 GMTs were non-inferior in groups 2, 3, and 4 and were superior in group 2. Six month post-dose 3 GMTs were superior in groups 2, 3, and 4 for each genotype, except HPV 18 in group 3. Age-adjusted post dose 2 titers were significantly lower than post-dose 3 titers when dose 2 was on time but were significantly higher when dose 2 was substantially late. Participants ≥15 years old had no difference in post-dose 2 titers compared to <15 year olds when dose 2 was substantially delayed.

Conclusions—Prolonged intervals between doses do not appear to diminish and may enhance antibody response to 4vHPV.

Keywords

Human papillomavirus; Vaccine; Immunity; Geometric mean titers; Dosing; Interval

1. Introduction

In the United States (US), human papillomavirus (HPV) vaccination was recommended for girls in 2006 as a 3-dose series for 9 to 26 years old with the second dose administered one to two months after the first dose and the third dose six months after the first dose[1, 2].

Recently, recommendations in the US were modified to include a 2-dose schedule given 6-12 months apart for adolescents initiating the vaccine series at 9-14 years[3].

Delays between doses are common, with rates of on-time completion declining over time since licensure[4]. Two reviews[5, 6] of clinical populations indicate that the average intervals are approximately six months between doses 1 and 2 and 11 months between doses 2 and 3. Few studies have examined HPV vaccine immunogenicity when given at prolonged intervals in the community setting[7].

With less than half of adolescent females completing the series within the recommended dosing schedule, we sought to better understand implications for alternate dosing schedules. A large, multisite study was undertaken to examine antibody response to HPV types 6, 11, 16, and 18 among girls receiving the quadrivalent HPV vaccine (4vHPV) as part of routine health care. Because substantial delays between vaccine doses occur in the community, the effects of substantial delay in obtaining dose 2, dose 3, and both dose 2 and dose 3 were evaluated at one month and six months after receipt of dose 3 thus extending our understanding of HPV vaccination by examining predefined groups with more substantive delays and measuring persistence of antibody at six months. [7]. Due to interest in 2-dose schedules, effects of on-time and substantial delay in obtaining dose 2 were evaluated at four months after receipt of dose 2 in a subset of participants. Lastly, the frequency and impact of vaccination with other vaccines at the time of 4vHPV on immune response was assessed.

2. Methods

2.1 Participants

This multisite prospective study enrolled healthy girls aged 9 to 17 years who received the 4vHPV as part of routine clinical care from pediatric and adolescent practices in Ohio, Tennessee, Georgia, Missouri, Pennsylvania, Maryland, and Washington between June 2010 and August 2013. Girls provided written assent; parents/legal guardians provided informed consent. In Ohio and Tennessee, if a parent/legal guardian was not available to provide consent, parental consent was waived after three attempts to contact the parent. Girls were excluded if they had received any HPV vaccine other than 4vHPV, blood, blood products, immunosuppressive medications in the past three months, a live virus vaccine (not including live attenuated influenza virus vaccine) within four weeks before or after the third 4vHPV dose, or had a history of malignancy or a confirmed or suspected immunodeficiency. Other criteria are detailed on [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00524745) (NCT00524745).

2.2 Design

The study was approved by each site's Institutional Review Board. Eligible girls were enrolled either the day of and prior to receiving their third 4vHPV dose or up to 28 days after receipt of dose 3. Upon enrollment, participants were assigned to a study group defined by the intervals between booster doses. Intervals were calculated from dates of vaccination obtained from clinical medical records and patient's immunization cards. Participants in group 1 (control group) received dose 2 on time (within 51-70 days of dose 1) and dose 3 on time (within 106-137 days of dose 2). Group 2 received dose 2 on time and dose 3

substantially late (≥ 240 days after dose 2). Group 3 received dose 2 substantially late (≥ 120 days after dose 1) and dose 3 on time. Group 4 received dose 2 substantially late and dose 3 substantially late. Participants receiving doses at intervals not included in the groups described above were included in group 5; these participants were included in analyses of characteristics and concomitant vaccines. Definitions for on-time and substantially late administration were based on previous studies, 4vHPV package insert, and Centers for Disease Control and Prevention Advisory Committee on Immunization Practices recommendations for HPV immunization [5] [8, 9].

Participants in each group had blood drawn for measurement of antibody titers one month (approximately 28 days; range 21-35 days) and six months (approximately 183 days; range 176-190 days) after dose 3. Participants in groups 1 and 3 who enrolled prior to receiving dose 3 had blood drawn four months (approximately 123; range 106-137 days) after dose 2. This permitted analyses of post dose 2 titers among participants with on-time and substantially late dose 2. Vaccine was supplied by clinical sites; vaccination was not a study procedure.

2.3 Study procedures

Upon enrollment, participant's demographic and medical information were obtained from participants and parent/legal guardian. Immunization history was obtained from participants' medical record. Serum was collected at designated time points, frozen and shipped to a central storage facility until transported to the reference laboratory for antibody testing.

2.4 Antibody assays

Antibody responses to 4vHPV genotypes were measured using a multiplex ELISA (M4ELISA)[10] performed at the Centers for Disease Control and Prevention HPV Immunology Laboratory. Briefly, it is a direct virus-like particle based IgG ELISA on Meso Scale Discovery platform. Serial dilutions of the test and reference sera and the parallel line (PLL) method were used to determine the amount of antibody present in the test serum relative to the standard serum. The threshold for seropositivity for each HPV type was calculated as equal to or above the 99th percentile of Johnson-Su probability distribution of children's sera signal run at 1:100 (gift from Dr. Joakim Dillner, Lund University, Sweden). Titers were reported in Arbitrary Units/ml (AU/ml) for HPV 6 and 11 and in International Units/ml (IU/ml) for HPV 16 and 18.

2.5 Statistics

The primary study objective was to compare immunogenicity one month after completing the 3-dose vaccination series when the second and/or third vaccination dose was received at substantially prolonged intervals to immunogenicity when both the second and third dose were received within or close to the recommended intervals. Based on predetermined power calculations to estimate sample sizes, this study aimed to enroll 211 participants in the control group and investigational groups 2, 3 and 4. Sample size was estimated using the non-inferiority test for each study group and HPV type along with estimates of the distribution of one month post dose 3-vaccination titers [11]. Calculations accounted for multiple comparisons.

For each study group, GMTs and 95% confidence intervals (CIs) for each HPV type were calculated. GMTs were age adjusted by determining the least square (LS) GMTs and 95% (CI) by regression analysis using log10 GMT as the response and continuous age as the predictor. GMTs and LS GMTs were similar and LS GMTs were used to determine inferiority and superiority, so only LS GMTs are presented.

Non-inferiority and superiority were calculated using the difference in LS GMTs of the control and a study group and the associated two-sided 97% confidence interval (CI) (97% CI was used to adjust for the three pairwise comparisons). A study group LS GMTs for an HPV type was considered non-inferior to the control group if the upper limit of the 97.5% CI was < 0.176 (equivalent to a GMT of the control/GMT of the study group < 1.5 if a single pairwise comparison was made). A study group LS GMTs for an HPV type was considered superior to the control group if the upper limit of the 97.5% CI was < 0 . To determine the effect of delaying dose 2, GMTs were calculated, as described above, for post-dose 2 titers, using a 90% CI. Two post hoc analyses were completed to further evaluate post-dose 2 titers. To compare antibody responses after 2 doses versus 3 doses, post-dose 2 and 6 month post-dose 3 GMTs were calculated for titers from participants in groups 1 and 3. Post dose 2 GMTs were measured approximately 4 months after dose 2, therefore, 6-month, rather than 1-month, post dose 3 GMTs were used in these analyses to more closely match the time from vaccination to antibody measurement. To compare antibody responses in < 15 year olds (currently recommended for 2 dose vaccine schedule) and 15 year olds (currently recommended for 3 dose schedule) among those with on time and substantially late dose 2, post dose 2 titers among group 1 and group 3 were stratified by age and GMTs calculated. Differences in titers were tested for significance using ANOVA.

Descriptive analyses were performed to explore the magnitude of GMTs in groups with concomitant vaccinations at HPV dose 2 and HPV dose 3. Analysis software was SAS V9.3.

3. Results

A total of 1,321 adolescents were enrolled: 224 in the control group (group 1; on-time doses 2 and 3), 173 in group 2 (on-time dose 2, substantially late dose 3), 222 in group 3 (substantially late dose 2, on-time dose 3), 235 in group 4 (substantially late doses 2 and 3), and 467 in group 5 (participants not meeting criteria for groups 1 through 4). As shown in Figure 1, a total of 1,260 (95%) participants had one month and 1119 (85%) had six month post dose 3 titers measured. Of the 156 (11.8%) participants who terminated early from the study, loss to follow-up was the most common reason (84 adolescents [6.4%]).

The median age of participants was 14 years (range 10-17). Baseline characteristics are shown in Table 1. Differences between groups included the control group which had a higher proportion of white participants (53.6%) compared to other groups (42.2%) and group 4 had a higher median age (15 years) and a higher median body mass index (24 mm/kg²) compared to other groups (14 years and 22-23 mm/kg² respectively). The intervals between doses are shown in Table 2.

3.1 Antibody responses one month after dose 3

One month after dose 3, all participants were seropositive to all four HPV types. GMTs for HPV 6, 11, 16, and 18 in groups 2-4 were non-inferior to the control group. As shown in Table 3, titers for HPV 6, 11, 16, and 18 in group 2 and HPV 11 in group 3 were significantly higher than the control group.

3.2 Antibody responses six months after dose 3

Six months after dose 3, all participants were seropositive to all four HPV types, except one individual to HPV 18. GMTs for each HPV type in groups 1-4 had decreased to less than one-half the titers observed at one month (Table 3). Similar to results at one month, titers at six months for HPV 6, 11, 16, and 18 in groups 2-4 were higher than and non-inferior to the control group. Titers for HPV 6, 11, and 16 in groups 2, 3, 4, and 5 were superior to the control group; titers for HPV 18 in groups 2, 4, and 5 were also superior compared to controls. Group 2 continued to have the highest HPV titers for each HPV type compared to other study groups.

3.3 Effect of delaying dose 2

A total of 390 participants had titers measured before dose 3 (approximately four months after dose 2): 192 in group 1 and 198 in group 3. Each participant was seropositive after dose 2 to all four HPV types. Table 4 shows the effect of delaying dose 2. Post dose 2 GMTs in group 3 (substantially late dose 2) were non-inferior and superior to group 1 (on time dose 2) with GMTs 2.5 to 5 times higher in the substantially late dose 2 group.

3.4 Antibody responses after 2 versus 3 doses

Table 5 shows the comparison of titers after two versus three doses in participants in groups 1 and 3 who had both post-dose 2 and post-dose 3 titers. Of note, post-dose 2 titers were measured 106-137 days after dose 2 while post-dose 3 titers were obtained after a longer interval (174-233 days). When doses 2 and 3 were received on time (n=182), post dose 2 GMTs for all four HPV types were significantly lower ($p<.001$), 40%-50% lower, than post-dose 3 GMTs. When dose 2 was received substantially late and dose 3 received on time (n=178), post dose 2 GMTs for HPV 6, 11, and 16 were significantly higher by 16-32% ($p<.001$) than post-dose 3 GMTs. There was no difference in GMT of HPV 18 after dose 2 compared to dose 3.

3.5 Antibody responses after 2 doses in younger and old girls

Table 6 shows comparison of unadjusted post-dose 2 GMTs in <15 versus ≥15 year olds in groups 1 and 3. When dose 2 was on time, GMTs of HPV 6 and 18 were significantly higher among <15 year olds compared to ≥15 year olds. When dose 2 was substantially late, titers observed in <15 year olds were not significantly different from titers in ≥15 year olds among dosing intervals examined.

3.6 Concomitant vaccinations

Overall, 471 (35.7%) participants received one or more vaccines on the same day as HPV dose 2, and 410 (31.0 %) participants received one or more vaccines on the same day as

HPV dose 3. The most common concomitant vaccinations received were influenza (18.4% with dose 2, 17% with dose 3), hepatitis A (10.2% with dose 2, 10.8% with dose 3), meningococcus (9.5% with dose 2, 5.5% with dose 3), and tetanus, diphtheria, and acellular pertussis (5.8% with dose 2, 3.9% with dose 3). Within each study group, GMTs of groups receiving a concomitant vaccine with dose 2 or dose 3 were similar to GMTs of groups with no concomitant vaccines for all of the four HPV types (data not shown).

4. Discussion

This study was initiated to examine immunogenicity of 4vHPV in a clinic-based population of young women receiving routine care when doses were received at non-recommended intervals, specifically at substantially prolonged intervals for dose 2 and/or dose 3. We also examined immunogenicity after two 4vHPV doses. When intervals between 4vHPV doses were substantially delayed, all four antigens (HPV 6, 11, 16, and 18) were immunogenic at each dosing interval. Without exception, GMTs for all four antigens in groups with substantially prolonged intervals were non-inferior to GMTs in the control group with on-time dose 2 and dose 3. With the exception of HPV 18, titers at one and six months after dose 3 were higher in groups with substantially prolonged intervals compared to the control group. Differences were greater at six months. Six months after dose 3, all GMTs in the three delayed investigational groups, except for HPV 18 in group 3, were significantly higher than the control group. GMTs six months after three doses were highest among those with on-time dose 2 and substantially delayed dose 3. This suggests a second dose within two months of the first dose combined with a delayed third dose elicits the most robust immune response sustained at least 6 months following the last dose.

In randomized trials of HPV vaccination[12, 13], prolonged intervals between doses did not significantly decrease the antibody response to each HPV vaccine type and often resulted in higher antibody titers. For example, a study in college-aged students demonstrated that HPV4 given according to a 0-, 2-, and 12-month schedule compared to the recommended dosing schedule[13] and schedules that included the third immunization delivered as late as 24 months after the first dose were shown to be non-inferior to standard dosing regimens[12]. Since vaccine licensure, substantial delays in vaccine schedules have been common in the community. In this study, we found that delays of 120 days for dose 2 and 240 days for dose 3 were not detrimental and in fact, prolonged intervals improved the antibody response. These increases remained at six months after receipt of dose 3, thereby providing reassuring data that not only the peak but also the duration of the antibody response are not adversely impacted by prolonged delays. These findings support current recommendations that interrupted HPV vaccination schedules do not need to be restarted[1].

This study was also designed to evaluate the antibody response after two 4vHPV doses. Antibody responses to all four antigens after two doses were superior when the second dose was delayed compared to when the second dose was on time. These outcomes are consistent with findings from Russell et al[7] and are supported by comparisons of immunogenicity after two doses given at six month vs 12 month intervals[14, 15]. These findings support recent recommendations for the 2-dose regimen in 9-14 year olds[3]. It is reassuring that responses are robust after a delayed dose 2 because delays in receiving dose 2 occur

frequently, as was seen in our study and as previously reported for the three-dose schedules[5, 6].

Antibody responses after on time dose 2 were substantial, but 40-50% lower than after dose 3. Conversely, among those with a delayed dose 2 and on time dose 3, the antibody response after dose 2 was 16-32% higher than after dose 3. The higher boost after a delayed dose 2 compared to an on-time dose 3 may be explained by a greater immune response after a longer interval between dose 1 and 2 (median 372, range 120-1975 days) and shorter time to titer measurement (106-137 days) compared to dose 3 (median interval 123, range 106-137 days; titers measured 174-233 days). The durability of the response after dose 2 could not be measured using our study design.

Trials comparing two dose schedules compared with three dose schedules have supported approval of a two dose schedule in 9 through 14 year olds[15–20]. Compared to antibody responses after dose 2 in younger girls (<15 years), antibody responses in older girls (15 years) in this study were not significantly different when dose 1 and 2 were received at a substantially long interval, suggesting that a 2 dose schedule might be adequate for older girls. Further research is warranted to assess a 2 dose schedule in older girls. Additionally, GMTs of four HPV vaccine types were similar between groups receiving a concomitant vaccine with dose 2 or dose 3 4vHPV compared to groups with no concomitant vaccines[21].

Strengths of this study include a study population with young girls recruited from multiple clinical sites around the US who received 4vHPV as part of routine health care. The study sample was estimated to be large enough to detect small differences between groups. Results of 4vHPV immunogenicity remain relevant as 4vHPV was most commonly used through 2015, prior to introduction of the nonavalent HPV vaccine. Among those with delayed dose 2 and on time dose 3, higher antibody doses after delayed dose 2, compared to on-time dose 3 may be explained by both the longer intervals between dose 1 and 2 and the shorter time between dose 2 and titer measurement. Although protective antibody levels are thought to be quite low, the importance of different antibody levels is not known. Vaccine efficacy against disease was maintained for at least 48 months post-vaccination despite the loss of detectable antibodies to HPV 18 in about 40% of vaccine recipients[22].

These findings suggest that delays in administration of either dose, even 240 days for dose 3, will not negatively affect the protection offered by HPV vaccination. The results of this trial also support recent recommendations of the 2-dose regimen in 9-14 year olds.

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Abbreviations

CI	confidence interval
ELISA	enzyme-linked immunosorbent assay
GMT	geometric mean titer
HPV	human papillomavirus
Log10	logarithmic base 10
LS	least squares
4vHPV	quadrivalent human papillomavirus vaccine
US	United States
VTEU	Vaccine and Treatment Evaluation Unit

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Figure 1. Number of participants with immunogenicity results

Abbreviations: OT/OT, both doses on time; OT/SL, dose 2 on time/dose 3 substantially late; SL/OT, dose 2 substantially late/dose 3 on time; SL/SL, both doses substantially late; LTF, lost to follow-up; NC, noncompliance; WP, withdrawn by parent; WI, withdrawn by investigator; NV, enrolled but not vaccinated.

¹ Participants were enrolled either before or after HPV vaccine dose 3

² Participants in Group 1 and Group 3 who enrolled before receiving HPV vaccine dose 3 had a pre-dose 3 blood draw

³ Data has been combined for simplicity as no significant differences were detected between groups for any period

⁴ The number of participants at each analysis point does not add up to the total participant numbers in the group because not all participants were available at all visits. Some participants had data from the 1 month post dose 3 but not the 6 month post dose 3 visit; other participants had data from the 6 month post dose 3 visit but not the 1 month post dose visit.

Table 1

Characteristics of Participants at the Baseline Visit by Study Group

Characteristic	All Participants N=1321	Both Doses on Time ^a (Control) n=224	Dose 2 on Time Dose 3 Substantially Late ^b (Group 2) n=173	Dose 2 Substantially Late, Dose 3 on Time ^c (Group 3) n=222	Both Doses Substantially Late ^d (Group 4) n=235	Other Intervals ^e (Group 5) n=467	p-Value
Age (y), mean (SD) [range]	14.1 (1.76) [10–17]	13.7 (2.00) [10–17]	14.2 (1.78) [10–17]	13.9 (1.60) [11–17]	14.7 (1.49) [11–17]	14.2 (1.77) [10–17]	<.001 ^f
Race, n (%)							
White	521 (39.4)	120 (53.6)	68 (39.3)	74 (33.3)	62 (26.4)	197 (42.2)	
Black	673 (50.9)	79 (35.3)	84 (48.6)	123 (55.4)	155 (66.0)	232 (49.7)	<.001 ^h
Multi-racial	97 (7.3)	17 (7.6)	19 (11.0)	23 (10.4)	15 (6.4)	23 (4.9)	
Other ^g	30 (2.3)	8 (3.6)	2 (1.2)	2 (0.9)	3 (1.3)	15 (3.2)	
Non-Hispanic or non-Latina, n (%)	1250 (94.6)	208 (92.9)	162 (93.6)	211 (95.0)	224 (95.3)	445 (95.3)	0.66 ^h
Body mass index ⁱ							
Mean (SD) [range]	24.4 (6.49) [13–57]	23.3 (5.99) [14–48]	24.3 (6.72) [15–57]	23.7 (5.79) [14–43]	25.6 (6.96) [15–56]	24.7 (6.60) [13–57]	.001 ^f

Abbreviation: NA, not applicable.

^aDose 2, 51–70 days after dose 3; dose 3, 106–137 days after dose 2.^b240 Days after dose 2.^c120 Days after dose 1.^dDose 2, 120 days after dose 1; dose 3, 240 days after dose 2.^eAny other interval not included in groups 1–4.^fAnalysis of variance.^gIncludes Asian, American Indian/Alaska Native, and unknown.

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χ^2 Chi-square test.

i Calculated as weight in kilograms divided by height in meters squared.

Table 2

Days Between 4vHPV Doses by Study Group

Interval	Both Doses on Time ^a (Control)	Dose 2 on Time Dose 3 Substantially Late ^b (Group 2)	Dose 2 Substantially Late Dose 3 on Time ^c (Group 3)	Both Doses Substantially Late ^d (Group 4)	Other Intervals ^e (Group 5)
	n=224	n=173	n=222	n=235	n=467
Days: mean, median (range)					
Doses 1 and 2	62.9, 63 (52-70)	62.9, 63 (51-70)	472.1, 372 (120-1975)	438.6, 392 (122-1106)	227.7, 101 (28-1678)
Doses 2 and 3	123.9, 124 (106-137)	501.4, 381 (240-1893)	121.9, 123 (106-137)	498.1, 392 (241-1251)	226.1, 169 (47-1593)

^a Dose 2, 51-70 days after dose 3; dose 3, 106-137 days after dose 2.^b 240 Days after dose 2.^c 120 Days after dose 1.^d Dose 2, 120 days after dose 1; dose 3, 240 days after dose 2.^e Any other interval not included in groups 1-4.

Table 3

Antibody responses one month and six months after dose 3 and comparison of groups with substantially delayed doses to the control group

HPV Type	Both Doses on Time ^a	Dose 2 on Time Dose 3 Substantially Late ^b	Dose 2 Substantially Late ^c Dose 3 on Time	Both Doses Substantially Late ^d
	(Control)	(Group 2)	(Group 3)	(Group 4)
One month after dose 3	n=219	n=162	n=212	n=219
HPV 6				
LS means of GMTs ^e	239.9	490.23	258.1	253.6
95% CI of LS means	212.9, 270.4	427.1, 562.8	228.7, 291.3	224.8, 286.1
97% CI of difference in LS means ^{e,f}		-0.40, -0.22 ^{*,†}	-0.11, 0.05 [*]	-0.11, 0.06 [*]
HPV 11				
LS means of GMTs ^e	271.2	666.6	337.6	318.0
95% CI of LS means	241.2, 304.8	582.6, 763.0	299.9, 380.0	282.6, 357.8
97% CI of difference in LS means ^{e,f}		-0.48, -0.30 ^{*,†}	-0.17, -0.02 ^{*,†}	-0.15, 0.01 [*]
HPV 16				
LS means of GMTs ^e	1026.0	1899.4	1119.2	1101.9
95% CI of LS means	918.2, 1146.4	1671.1, 2159.1	1000.4, 1252.2	985.3, 1232.4
97% CI of difference in LS means ^{e,f}		-0.35, -0.19 ^{*,†}	-0.11, 0.04 [*]	-0.11, 0.05 [*]
HPV 18				
LS means of GMTs ^e	362.2	648.3	344.5	390.5
95% CI of LS means	317.2, 413.5	556.3, 755.5	301.2, 393.9	341.7, 446.4
97% CI of difference in LS means ^{e,f}		-0.35, -0.16 ^{*,†}	-0.07, 0.11 [*]	-0.12, 0.06 [*]
Six months after dose 3	n=200	n=142	n=191	n=194
HPV 6				
LS means of GMTs ^e	62.6	184.2	90.9	100.1
95% CI of LS means	53.8, 72.8	154.1, 220.2	77.9, 106.0	85.8, 116.8
97% CI of difference in LS means ^{e,f}		-0.58, -0.36 ^{*,†}	-0.27, -0.06 ^{*,†}	-0.31, -0.10 ^{*,†}
HPV 11				
LS means of GMTs ^e	70.9	236.9	128.8	127.5
95% CI of LS means	61.4, 81.9	199.8, 280.9	111.2, 149.2	110.1, 147.8
97% CI of difference in LS means ^{e,f}		-0.63, -0.42 ^{*,†}	-0.36, -0.16 ^{*,†}	-0.36, -0.15 ^{*,†}
HPV 16				
LS means of GMTs ^e	311.1	858.3	439.7	491.7
95% CI of LS means	272.3, 355.5	733.3, 1004.6	383.7, 503.7	429.1, 563.5
97% CI of difference in LS means ^{e,f}		-0.54, -0.34 ^{*,†}	-0.24, -0.06 ^{*,†}	-0.29, -0.11 ^{*,†}
HPV 18				

HPV Type	Both Doses on Time ^a	Dose 2 on Time Dose 3 Substantially Late ^b	Dose 2 Substantially Late ^c Dose 3 on Time	Both Doses Substantially Late ^d
	(Control)	(Group 2)	(Group 3)	(Group 4)
LS means of GMTs ^e	84.9	231.1	100.4	135.6
95% CI of LS means	71.8, 100.4	189.6, 281.8	84.6, 119.2	114.3, 161.0
97% CI of difference in LS means ^{e,f}		-0.56 -0.31 ^{*+}	-0.19 0.04 [*]	-0.32 -0.09 ^{*+}

Abbreviations: HPV, human papillomavirus; LS, least squares; GMT, geometric mean titer; NA, not applicable because of heterogeneity of intervals in Group 5.

^aDose 2, 51-70 days after dose 1; dose 3, 106-137 days after dose 2.

^bDose 2, 51-70 days after dose 1; dose 3, 240 days after dose 2.

^cDose 2, 120 days after dose 1; dose 3, 106-137 days after dose 2.

^dDose 2, 120 days after dose 1; dose 3, 240 days after dose 2.

^eLS means indicates least squares means from a regression analysis using the log 10 titer response, adjusting for age.

^fLS mean difference indicates least squares mean of the difference in the Control (Group 1) and Investigative group from a regression analysis using the log 10 titer response, adjusting for age. 97% CI of difference of LS means indicates 97% confidence interval of the LS mean difference, which is adjusted for the three pairwise comparisons.

^{*}Non-inferiority = upper limit of 97% CI < 0.176 demonstrated.

⁺Superiority = upper limit of 97% CI < 0 demonstrated.

Bold indicates superiority demonstrated.

GMTs are reported in Arbitrary Units/ml (AU/ml) for HPV 6 and 11 and in International Units/ml (IU/ml) for HPV 16 and 18.

Table 4

Effect of delaying dose 2: Antibody responses after dose 2 among those substantially late for dose 2 compared to those on time for dose 2

HPV Type	On-Time Dose 2 ^a Group 1	Substantially Late Dose 2 ^b Group 3
	n=192	n=198
HPV-6		
LS means of GMTs ^c	30.4	115.8^{*+}
90% CI of LS means	26.5, 34.8	101.3, 132.4
90% CI of difference in LS means ^d		-0.66, -0.50^{*+}
HPV-11		
LS means of GMTs ^c	40.6	197.0^{*+}
90% CI of LS means	35.6, 46.3	173.4, 224.6
90% CI of difference in LS means ^d		-0.77, -0.61^{*+}
HPV-16		
LS means of GMTs ^c	181.4	557.4^{*+}
90% CI of LS means	160.3, 205.3	493.4, 629.8
90% CI of difference in LS means ^d		-0.56, -0.41^{*+}
HPV-18		
LS means of GMTs ^c	42.0	105.4^{*+}
90% CI of LS means	36.8, 48.1	92.4, 120.3
90% CI of difference in LS means ^d		-0.48, -0.32^{*+}

Abbreviation: HPV, human papillomavirus; LS, least squares; GMT, geometric mean titer.

^aParticipants in group 1 with post-dose 2 titers; dose 2 received 51-70 days after dose 1.

^bParticipants in group 3 with post-dose 2 titers; dose 2 received 240 days after dose 1.

^cLS means indicates least squares means from a regression analysis using the log 10 titer response measured approximately four months after dose 2, adjusting for age.

^dLS mean difference indicates least squares mean of the difference in the Control (Group 1) and Investigative group from a regression analysis using the log 10 titer response, adjusting for age; 90% CI of difference of LS means.

^{*}Non-inferiority = upper limit of 90% CI < 0.176 demonstrated.

⁺Superiority = upper limit of 90% CI < 0 demonstrated.

Bold indicates superiority demonstrated.

GMTs are reported in Arbitrary Units/ml (AU/ml) for HPV 6 and 11 and in International Units/ml (IU/ml) for HPV 16 and 18.

Table 5

Comparison of post dose 2 and post dose 3 titers when dose 2 received on time or substantially late and dose 3 received on time

On-Time Dose 2, On-Time Dose 3 ^a (n=182)					Substantially Late Dose 2, On-Time Dose 3 ^b (n=178)				
Group 1					Group 3				
HPV Type	Post-dose 2 ^c		Post-dose 3 ^d		Post-dose 2 ^c		Post-dose 3 ^d		
HPV 6									
LS means of GMTs ^e	30.9		62.0		112.0		91.9		
95% CI of LS means	26.5, 36.1		53.1, 72.3		94.5, 132.0		77.8, 108.7		
p-Value ^f			<0.001				<0.001		
HPV 11									
LS means of GMTs ^e	41.7		69.4		190.2		130.2		
95% CI of LS means	35.8, 48.4		59.7, 80.7		161.9, 223.6		110.8, 153.0		
p-Value ^f			<0.001				<0.001		
HPV 16									
LS means of GMTs ^e	183.7		307.9		535.5		449.3		
95% CI of LS means	159.5, 211.7		267.2, 354.8		457.4, 626.9		383.8, 526.0		
p-Value ^f			<0.001				<0.001		
HPV 18									
LS means of GMTs ^e	41.9		87.5		102.7		104.8		
95% CI of LS means	35.6, 49.5		74.2, 103.2		86.6, 121.9		88.3, 124.3		
p-Value ^f			<0.001				0.832		

Abbreviation: HPV, human papillomavirus; LS, least squares; GMT, geometric mean titer.

^aParticipants in group 1 with post-dose 2 titers; dose 2 received 51-70 days after dose 1.

^bParticipants in group 3 with post-dose 2 titers; dose 2 received 240 days after dose 1.

^cSamples obtained 106-137 days after dose 2.

^dSamples obtained 174-233 days after dose 3.

^eLS mean indicates least-squares means from a regression analysis using the log 10 titer response, adjusting for age.

f_j Analysis of variance testing whether the change of titer values from dose 2 to dose 3 is different between ages. GMTs are reported in Arbitrary Units/ml (AU/ml) for HPV 6 and 11 and in International Units/ml (IU/ml) for HPV 16 and 18.

Table 6

Comparison of antibody responses after dose 2 in <15 year olds versus 15 year olds when dose 2 received on-time or substantially late

HPV Type	On-Time Dose 2 ^a			Substantially Late Dose 2 ^b		
	<15 years (n=120)	15 years (n=62)	p-value ^d	<15 years (n=121)	15 years (n=57)	p-value ^d
HPV-6						
Means of GMT ^c	35.7	23.4	0.016	115.5	103.9	0.58
95% CI	29.3, 43.6	17.8, 30.9		93.1, 143.3	75.8, 142.3	
HPV-11						
Means of GMT ^c	44.4	36.8	0.26	194.8	181.0	0.69
95% CI	36.7, 53.7	28.2, 48.0		158.1, 240.0	133.5, 245.3	
HPV-16						
Means of GMT ^c	197.5	159.7	0.17	574.5	461.2	0.23
95% CI	165.5, 235.6	125.0, 204.2		468.9, 703.9	343.0, 620.1	
HPV-18						
Means of GMT ^c	50.0	29.8	0.002	107.7	92.8	0.43
95% CI	41.4, 60.4	22.9, 38.8		87.2, 133.1	68.2, 126.3	

Abbreviation: HPV, human papillomavirus. GMT, geometric mean titer. CI, confidence interval.

^aParticipants in group 1 with post-dose 2 titers; dose 2 received 51-70 days after dose 1.

^bParticipants in group 3 with post-dose 2 titers; dose 2 received 240 days after dose 1.

^cSamples obtained 106-137 days after dose 2.

^dAnalysis of variance to test differences in titer values in those <15 years and those 15 years old.

GMTs are reported in Arbitrary Units/ml (AU/ml) for HPV 6 and 11 and in International Units/ml (IU/ml) for HPV 16 and 18.