Persistence of Immunity When Using Different Human Papillomavirus Vaccination Schedules and Booster-Dose Effects 5 Years After Primary Vaccination

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Background. There are limited data regarding the duration of immunity induced by different human papillomavirus (HPV) vaccination schedules and the immunogenicity of a booster dose of both bivalent HPV vaccine (bHPV) or quadrivalent HPV vaccine (qHPV).

Methods. Follow-up of a nonrandomized clinical trial to evaluate the 5-year antibody persistence of the bHPV in girls (age, 9–10 years) and women (age, 18–24 years). Noninferiority of the 2-dose versus 3-dose schedule among girls was evaluated at months 54 (n = 639) and 64 (n = 990). Girls vaccinated with a 2-dose schedule of bHPV or qHPV received a booster dose of either vaccine at month 61. Immunogenicity was measured using a virus-like particle–based enzyme-linked immunosorbent assay. Geometric mean titers (GMTs) for HPV16/18 were estimated after stratification by vaccination schedule and age group.

Results. At months 54 and 64, the 2-dose schedule remained noninferior to the 3-dose schedule. GMTs remained above natural infection levels across all age groups up to 64 months. After the booster, anti-HPV16/18 GMTs increased exponentially with the same pattern, regardless of vaccine administered. No safety concerns were identified with the booster dose.

Conclusions. A 2-dose schedule is highly immunogenic in girls, suggesting a high immune memory. Thus, a booster dose is likely to be unprofitable, considering the low global immunization coverage.

Clinical Trials Registration. NCT01717118

Keywords. HPV vaccine; 2-dose schedule; alternative regimens; immunogenicity; booster dose; geometric mean antibody titer; interchangeability.

Evidence from human papillomavirus (HPV) vaccines continues to emerge, demonstrating their high immunogenicity and efficacy against HPV infection, cervical precancer, and invasive cancer [1–3]. While the first HPV vaccine recommendations suggested a 3-dose scheme, in 2014, the World Health Organization (WHO) recommended the 2-dose regimen for immunocompetent girls between 9 and 14 years old [2]. This decision was based on immunogenicity studies that showed noninferiority of 2 doses of either HPV vaccine in girls aged 9–14 years as compared to 3 doses in young women [4–7].

However, several questions about this decision have been voiced in the recent literature. The main concern is that the WHO recommendation is based on comparison of the immune response between girls and women, which could be clouded by differences in immune maturation [8]. A recent meta-analysis suggests that this could be a possibility; pooling results of 2 studies of same-age girls revealed that the 2-dose schedule was inferior to the 3-dose schedule [8]. Similar concerns have been raised about long-term immunogenicity under the 2-dose regimen [9]. A recent randomized clinical trial showed that the 2-dose regimen in girls aged 9–14 years produced an immunogenic response noninferior to that observed with 3 doses in young women after 5 years of follow-up [10]; thus, age differences could still influence this finding. To solve this limitation, further noninferiority trials conducted in immunologically comparable age groups are needed.

Immune response monitoring of alternative schedules for HPV vaccine in girls has been largely restricted from 12 to 60 months of follow-up [10–12]. A close monitoring of the vaccinated girls is necessary to assess the durability of antibody levels and, more importantly, to understand the immunologic memory produced by the vaccine [13]. The immune correlates of vaccine protection are still uncertain [14]. Follow-up of young women for at least 10 years after vaccination has revealed that...
antibody titers among vaccine recipients remain above those with natural infection [15], and there is evidence that even low antibody titers can prevent persistent infections and subsequent cervical disease [16, 17].

In 2008, Mexico implemented an extended-dose schedule of HPV vaccine at the national level, with HPV vaccine administered at months 0 and 6 and a booster dose administered at month 60. The booster was proposed as a mean to increase coverage in the population, given economic constrains at the time of the first vaccination campaign [18]. There is limited evidence about the effect of a booster dose of either HPV vaccine. A recent study analyzed the effect of a booster dose 3 years after primary immunization, using the quadrivalent HPV vaccine (qHPV) [19]; antibody titers increased 1 month after administration, although the persistence of this effect after 1 month is still unclear. Also, the magnitude of the immune response induced by a booster dose in groups previously vaccinated with different vaccines remains unknown.

Beyond immunogenicity, a common discussion has focused on the interchangeability of bHPV and qHPV. Most studies have been restricted to the analysis of antibody levels among recipients the same type of vaccine, although in real life, vaccines are being used interchangeably over time. For example, Mexico undergoes yearly competitive purchases of vaccines at the federal level; as a result, during their schedule, girls could receive bHPV or qHPV. Few studies have analyzed the safety and the interchangeability of vaccines in girls in terms of antibody levels.

Taking advantage of this setting, we conducted an extension study in which Mexican girls and young women participating in 2 initial nonrandomized clinical trials were invited to participate in a study to evaluate the immunogenicity and safety induced by 2 and 3 doses of bHPV at 54 and 64 months after administration of the first dose. We also aimed to assess the noninferiority of antibody concentrations elicited by the 2-dose schedule in girls aged 9–10 years, compared with that induced by a 3-dose schedule (M 0,1,6) in the same age group. Secondary objectives were to compare the immune response following a booster dose of a bHPV or qHPV administered at month 61 to girls previously vaccinated with 2 doses of either HPV vaccine and to assess the interchangeability of bHPV and qHPV as a booster dose.

**METHODS**

**Study Design**

By means of an open-label, nonrandomized clinical trial, we evaluated the long-term immunogenicity of bHPV administered via different dosing schedules to participants who were followed for 64 months (hereafter referred as the bHPV trial) [7]. Additionally, as part of a secondary analysis to evaluate the effect of a booster dose, girls aged 9–10 years from the 2-dose arm who were original participants in either the bHPV trial or an analogous open-label, nonrandomized clinical trial of qHPV (hereafter referred to as the qHPV trial) [20] were randomized to receive a booster dose of either HPV vaccine. The design and eligibility criteria from the 2 nonrandomized clinical trials up to month 21 were presented elsewhere [7, 20].

**Procedures**

In the bHPV trial, during November 2009, we enrolled 1,500 healthy girls aged 9–10 years and 499 women aged 18–24 years [7]. Girls were recruited at 81 public primary schools, and women were recruited among users of a primary care facility in Cuernavaca, Mexico.

Participants in the first vaccination phase of the HPV trial were allocated to one of the following groups (Figure 1), all of whom received bHPV: (1) girls aged 9–10 years on an extended vaccination schedule (at months 0, 6, and 60), (2) girls aged 9–10 years on a standard vaccination schedule (at months 0, 1, and 6), women aged 18–24 years on a standard vaccination schedule (at months 0, 1, and 6), and women aged 18–24 years on a 2-dose schedule (at months 0 and 6). The original extended schedule cluster (ie, vaccination at months 0, 6, and 60) was twice as large as the standard schedule cluster (ie, vaccination at months 0, 1, and 6), to partition the extended schedule group into the following 2 subgroups 5 years after receipt of the first vaccine dose: those who would receive only 2 doses and those who would receive the booster dose of vaccine.

Both bHPV and qHPV trials were approved by the Institutional Review Board of the National Institute of Public Health of Mexico, with corresponding authorizations received annually (number 833). All women and parents/legal guardians of girls signed informed consent forms following their review of protocol procedures. Both trials were registered at the Federal Commission for Protection against Sanitary Risks of Mexico. The trials are registered in ClinicalTrials.gov (registration number NCT017117118). The trials were conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines.

As part of the follow-up procedures of the bHPV trial, a third follow-up wave was implemented at month 54. During the 54-month wave, a subset of participants who accepted to continue their participation were invited to provide serum samples. This visit allowed us to track the immunity before the booster dose and to update contact information necessary for the next visit.

The original extended-dose group (ie, vaccination at months 0, 6, and 60), described above as group 1, was divided into 3 comparison groups 60 months after the first dose administration. The purpose of this new allocation was to evaluate the immune response at different dose schedules, including the effect of a booster dose 5 years after primary immunization. The following 3 groups vaccination schedules were created:
2 doses of bHPV (at months 0 and 6), 2 dose of bHPV (at months 0 and 6) and a booster dose of bHPV (at month 61), and 2 doses of bHPV (at months 0 and 6) and a booster dose of qHPV (at month 61). Additionally, a group of girls from the qHPV trial who had received 2 doses of qHPV (at months 0 and 6) were included as an ancillary booster-dose group. These girls were assigned to receive a booster dose of bHPV.

Vaccine Administration and Serum Sampling

For the booster, we administered the 2 commercially available vaccines according to allocation arm: bHPV (HPV16/18 L1 virus-like particle [VLP] AS04-adjuvanted vaccine [Cervarix; GlaxoSmithKline Biologicals, Rixensart, Belgium]) [21] and qHPV (HPV6/11/16/18 vaccine [Gardasil, Merck, Whitehouse Station, NJ]) [22].

To assess the antibody response and persistence, blood samples were obtained 54 months after the first vaccination from the participants in the bHPV trial and 64 months after the first dose administration in all study groups (Figure 1), including participants who received a booster dose of either HPV vaccine. HPV16 and HPV18 antibodies were assessed using a VLP-based direct enzyme-linked immunosorbent assay (ELISA), which is the standard assay for immunogenicity of bHPV that measures polyclonal antibodies [23, 24]. To ensure comparability, all serological assays were performed in a reference laboratory at the University of Ghent, Belgium, where the staff was blinded to the study groups. Details of the reference determinations have been previously described [25]. The measurement of antibody levels was performed using the same assay at the same laboratory for all groups.

Safety Assessment

The study staff recorded safety profile assessments of local symptoms (ie, pain and redness at the injection site) and general symptoms (ie, fever, headache, fatigue, gastrointestinal symptoms and/or abdominal pain, arthralgia, myalgia, urticaria, and rash) 30 minutes after the administration of each vaccine and at the next scheduled appointment among subjects who were followed. Any serious adverse event was inquired and registered by the staff in each contact.

Statistical Analysis

The immunogenicity analysis was conducted according to protocol and comprised study participants who met all eligibility criteria, complied with the protocol procedures, received all vaccinations allocated, and had data available from the serological assessments.

The outcomes of interest included anti-HPV16 and anti-HPV18 antibody levels throughout the first 5 years of follow-up, by dose schedule and age group.

The cutoffs prespecified to indicate seropositivity according to the limit of detection of the assay were antibody titers of at least 8 ELISA units (EU)/mL for anti-HPV16 and at least 7 EU/mL for anti-HPV18. However, to improve the precision of the assay, a modification of seropositivity cutoff was made recently. The immunogenicity data from month 54 onward use the new cutoff of ≥19 EU/mL and ≥18 EU/mL for anti-HPV16 and anti-HPV18, respectively [26]. This change would imply...
an even larger percentage increase in seronegativity because a more conservative cutoff was established.

To evaluate the long-term kinetics of the immune responses across groups, we used geometric mean titers (GMTs) and 95% confidence intervals (CIs) of vaccine-type HPV16 and HPV18 obtained during serological measurements at months 54 and 64 after the first vaccine administration. A noninferiority evaluation was performed for the GMTs. If the upper limit of the 95% CI for the GMT ratio between the girls aged 9–10 years receiving 3 doses and the girls receiving 2 doses of the bHPV was <2.0, noninferiority was demonstrated. Our noninferiority margin was set in accordance with previous clinical trials that were designed to compare 2-dose to 3-dose schedules [4–6, 10–12, 14]. The statistical analyses were performed using Stata software, version 14.0 (Stata, College Station, TX).

RESULTS

The number of subjects throughout all study visits is shown in Figure 1. In the current report, we focus on evaluating the persistence of the antibody response from 54 months after the first vaccine dose and up to month 64 of follow-up. Of 1,266 participants in this follow-up (71.8% of the participants with antibody titers measured at month 21), 1,248 provided a serum sample for serological tests at either of the 2 visits (639 at month 54 and 990 at month 64). Girls and women who were invited to the 54-month visit were not identical to those measured at visit 64; an overlap of 376 subjects (30.0%) was observed among samples measured at both times.

Of 675 girls from the extended-schedule group (M 0,6,60) who were successfully reached, 237 received a booster dose of bHPV, 237 received a booster dose of qHPV, and the remaining 201 received only the 2-dose schedule (M 0,6). A set of 56 girls of the same age who previously received 2 doses of qHPV (M 0,6) during the qHPV trial received a dose of bHPV at this time point as a booster dose.

The median follow-up time from the date of first vaccination to the third visit was 54 months (range, 49–56 months.) The last immunogenicity visit reported in this analysis occurred at a median time of 64 months (range, 61–73 months) since the first dose was received. A high rate of seropositivity was observed across dosage groups for both HPV vaccine types, with 99.8% and 98% of participants having GMTs above the serostatus cutoff at months 54 and 64, respectively. Of the 639 participants with month 54 data, only 1 woman from the 2-dose group was seronegative for both HPV types. At month 64, this woman remained seronegative, and 1 girl who received the 2-dose schedule (M 0,6) had HPV16/18 antibody seroreversion at this last visit.

The GMTs at each time point and HPV type are presented in Table 1. GMTs against HPV16 and HPV18 decreased over time but persisted above natural infection levels across all vaccinated groups. A stable plateau was observed between months 54 and 64 after the first vaccine dose. By month 54, anti-HPV16 GMTs among girls who received the 3-dose

| Antibody | Girls Aged 9–10 y, by Vaccine Schedule | Women Aged 18–25 y, by Vaccine Schedule |
|----------|---------------------------------------|---------------------------------------|
| Anti-HPV16 | No. GMT, EU/mL (95% CI) Doses Received, No. GMT, EU/mL (95% CI) | GMT Ratio (95% CI) |
| 54 | 206 | 1312.17 (1177.17–1462.64) | 259 | 785.87 (703.73–877.59) | 1.67 (1.43–1.95) |
| 64 | 349 | 1191.64 (1094.72–1297.13) | 115 | 801.25 (660.41–972.13) | 1.49 (1.21–1.83) |
| 64 | 206 | 587.89 (519.36–665.46) | 259 | 356.11 (313.68–404.29) | 1.65 (1.38–1.97) |
| 64 | 349 | 546.24 (493.98–604.01) | 115 | 374.20 (303.88–460.80) | 1.46 (1.16–1.84) |
| 64 | 206 | 6430.14 (5816.52–7108.5) | 259 | 6531.83 (5855.65–7208.5) | |
| 64 | 349 | 6516.76 (4564.71–9517.29) | 115 | 6393.86 (5766.9–7063.5) | |
| 64 | 206 | 9244.65 (8369.97–10210.73) | 259 | 9244.65 (8369.97–10210.73) | |
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Abbreviations: CI, confidence interval; EU, enzyme-linked immunosorbent assay units; GMT, Geometric mean titers; M, month; 2b1q, 2 doses of bivalent HPV vaccine (M 0,6) and a booster dose (M 61) of quadrivalent HPV vaccine; 2q1b, 2 doses of quadrivalent HPV vaccine (M 0,2) and a booster dose (M 61) of bivalent HPV vaccine; 3b, 3 doses of bivalent HPV vaccine (M 0,6,61).
schedule (M 0,1,6; GMT, 1312.17 EU/mL [95% CI, 1177.17–1462.64]) were higher than those in the 2-dose group (GMT, 785.87 EU/mL [95% CI, 703.73–877.59]). However, the ratios of the GMT of the 2-dose group to that of the 3-dose group 54 months after the first dose was 1.67 for HPV16 and 1.65 for HPV18, with the upper limit of the 95% CI <2.0 for both ratios, demonstrating that the immune response of the 2-dose schedule was noninferior to that of the 3-dose schedule. The noninferiority criteria of the 2-dose schedule as compared to the 3-dose schedule were also met over the remaining study period.

The GMTs of HPV16 and HPV18 after the booster are presented in Table 1. An exponential increase in the GMT was observed after the booster dose (1–6 months), regardless of the vaccine administered. The anti-HPV16 GMT of the group that received a qHPV booster dose (15,905.1 EU/mL [95% CI, 14,199.5–17,815.6]) reached a GMT similar to that observed after the third dose (0.4–6.0 months) of bHPV among girls aged 9–10 years (18,219.19 EU/mL [95% CI, 16,832.97–19,719.57]) [7]. In contrast, the GMTs of both bHPV booster groups were lower than those in the 2-dose group (GMT, 785.87 EU/mL [95% CI, 703.73–877.59]). However, the ratios of the GMT of the 2-dose group to that of the 3-dose group 54 months after the first dose was 1.67 for HPV16 and 1.65 for HPV18, with the upper limit of the 95% CI <2.0 for both ratios, demonstrating that the immune response of the 2-dose schedule was noninferior to that of the 3-dose schedule. The noninferiority criteria of the 2-dose schedule as compared to the 3-dose schedule were also met over the remaining study period.

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slightly lower than those reported above, particularly for those who received qHPV as the primary immunization (Table 1). The kinetics of the HPV16 and HPV18 antibody response in all groups, according to dose schedule, are depicted in Figure 2.

Throughout the study follow-up, no serious adverse events or withdrawals related to the booster dose were reported. The most common local adverse event following the booster dose was pain in the injection site. The frequency of adverse events can be seen in Figure 3.

**DISCUSSION**

There is no immune metric, such as antibody concentration, that correlates with the protection afforded by HPV VLP vaccines. The available evidence is that, even in the absence of antibody as detected by the current seroassays [17], protection by one of the current vaccines against vaccine-type HPV disease (ie, cervical intraepithelial neoplasia 2/3) is maintained for at least 10–12 years [16]. The results of the follow-up phase of this clinical trial of the immunogenicity of bHPV in Mexican women revealed that a 2-dose vaccine schedule is safe and produced a robust immune response, with antibody levels that remained stable over 5 years after primary immunization for both HPV vaccine types. Moreover, we found that the antibody response of the 2-dose schedule was not inferior to the response observed in girls of the same age who received the standard 3-dose regimen over a 54- and 64-month follow-up period. These results are in line with previous evidence showing that the 2- and 3-dose schedules produce similar results in the same age group of girls [7, 27–29], supporting the current WHO recommendation of 2 doses. These results are relevant because immune comparability across different age groups has been expressed as a debatable argument for the 2-dose recommendation [8].

According to the kinetics of antibody levels from our study, the antibody response for the 2-dose schedule has good stability for up to 64 months and remains much higher than that after natural infection. This is consistent with previous findings reported by Romanowski et al, which showed that 2 doses of bHPV (spaced at 6 months) produced an immune response that persists for up to 5 years of follow-up [10]. Although our antibody responses differ slightly from those reported by Romanowski et al [10] and Huang et al [12], who used the same serological assay, our findings support evidence of the high and strong immune response in girls who received <3 doses. In comparing antibody response among different studies, it should be taken into account the difference in participants and study designs, as well as differences between laboratories and kit lots for VLP-based ELISAs.

Our results provide considerable insight into the interchangeability of bHPV and qHPV. To our knowledge, this is the first study providing information about the immunogenicity and safety of a qHPV or bHPV booster administered 5 years after the first dose to girls who had previously received 2 doses of bHPV. A previous study examined the interchangeability of HPV vaccines, but in girls who had received 2 doses of qHPV [19]. Our results show that the booster exponentially increases antibody titers to levels as high as those achieved 1 month after the second dose (M 0.6) in girls, independently of the type of vaccine (qHPV or bHPV). Our results are in line with those reported by Gilca et al, that either qHPV or bHPV as a booster provided a high antibody response, although in our case GMTs were higher in girls who received bHPV as a primary immunization (M 0.6) and received the qHPV booster, while in the study by Gilca et al GMTs were higher after receipt of a bHPV booster.

![Figure 3](image_url)

**Figure 3.** Proportion of adverse events among girls following a booster dose of bivalent human papillomavirus (HPV) vaccine (bHPV) or quadrivalent HPV vaccine (qHPV) administered 61 months after the first vaccine dose. The error bars represent 95% confidence intervals. There were no reports of paresthesia or erythema in the group of girls previously vaccinated with qHPV who received a booster of bHPV.
In this regard, although a direct comparison between our results and those of Gilca et al is limited because they used a different serological assay to measure the antibody response, it is likely, as Gilca et al suggested, that the differences are related to the higher immunogenicity of the ASO4-adjuvanted bHPV. The above findings could explain the higher magnitude of the antibody response among girls who received bHPV as the primary immunization in our study as compared to those vaccinated with qHPV. In a study reported by Einsten et al [30], bHPV produced significantly higher titers than qHPV, as well as more memory B cells, over 5 years of follow-up [31]. Similarly, a recent comparison between 2 doses of bHPV and 2 and 3 doses of qHPV among girls showed a higher immune response with bHPV after 36 months of follow-up [27]. However, beyond the adjuvant, it is unclear whether there are other vaccine characteristics that could explain the differential immune response [32].

Even so, the importance of the booster dose is questionable, as suggested by the findings of Scherer et al [31] and Gilca et al [19]. The evidence is that the priming dose of a vaccine dictates the memory response, and this is supported for qHPV in a study performed by Toh et al [28].

The interchangeability of bHPV and qHPV is important from a biological perspective, as well as from an economical perspective. In particular, countries that subject vaccine purchases to competitive processes can interchangeably buy qHPV or bHPV, knowing that the immune response will be comparable independently of the vaccine combination used during the schedule.

Some limitations must be mentioned. First, the lack of randomization in our study could have led to differential exposure to HPV, inducing differences in the immune response. However, this seems unlikely, especially in the group of girls involved in the noninferiority evaluation, given that the average age of first sexual intercourse in our population allows us to assume that most of them had not already been exposed to HPV infection (an important confounder for the immune response) at the time of vaccination but also at the time of booster dose administration. According to data from different national surveys in Mexico, <20% of women were sexually active before the age of 16 years [33]. Second, we cannot rule out a potential bias from withdrawal, because of the large loss to follow-up observed. The main reason for withdrawal was physical discomfort related to serum sample collection. Also, in the case of girls, the move from primary to secondary school represented one of the main challenges to follow-up. This limitation highlights the potential difficulty of administering a booster dose in a non–school-based immunization program 5 years after primary immunization.

The 2-dose regimen has enormous advantages from a public health perspective, including decreased costs, flexibility in the interval between doses, and an increase in vaccination coverage [34]. To date, the 2-dose schedule has been adopted in 65% of national immunization programs [1]. Currently, there is evidence that a single dose of HPV vaccine containing HPV16/18 induces a robust and sustained immune response [28, 35], and formal randomized clinical trials are being conducted to evaluate the protection afforded by a single dose of HPV vaccine [36, 37].

Given the concerns about the duration of immunity conferred by HPV vaccines, the long-term assessment of anti-HPV levels after a booster at year 5 offers compelling evidence to support the idea of the strong induction of memory B cells responsible for long-lasting humoral response. A close monitoring of the girls who had received different schedules of HPV vaccines will be relevant to confirm the long-term durability of the immune response.

Notes

Disclaimer. To allow for comparisons to results of previous studies, GlaxoSmithKline (GSK) Biologicals (Rixensart, Belgium) provided the immunogenicity testing. GSK had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.

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References

1. Brotherton JML, Bloem PN. Population-based HPV vaccination programmes are safe and effective: 2017 update and the impetus for achieving better global coverage. Best Pract Res Clin Obstet Gynaecol 2018; 47:42–58.
2. World Health Organization. Human papillomavirus vaccines: WHO position paper. http://www.who.int/wer/2014/wer8943.pdf?ua¼1. Accessed 23 March 2017.
3. Luostarinen T, Apter D, Dillner J, et al. Vaccination protects against invasive HPV-associated cancers. Int J Cancer 2018; 142:2186–7.
4. Romanowski B, Schwarz TF, Ferguson LM, et al. Immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine administered as a 2-dose schedule compared with the licensed 3-dose schedule: results from a randomized study. Hum Vaccin 2011; 7:1373–86.
5. Romanowski B, Schwarz TF, Ferguson LM, et al. Immune response to the HPV-16/18 AS04-adjuvanted vaccine administered as a 2-dose or 3-dose schedule up to 4 years after vaccination: results from a randomized study. Hum Vaccin Immunother 2014; 10:1155–65.
6. Dobson SR, McNeil S, Dionne M, et al. Immunogenicity of 2 doses of HPV vaccine in younger adolescents vs 3 doses

2 Doses and a Booster Dose of HPV Vaccines • JID 2019:219 (1 January) • 47
in young women: a randomized clinical trial. JAMA 2013; 309:1793–802.

7. Lazcano-Ponce E, Stanley M, Muñoz N, et al. Overcoming barriers to HPV vaccination: non-inferiority of antibody response to human papillomavirus 16/18 vaccine in adolescents vaccinated with a two-dose vs. a three-dose schedule at 21 months. Vaccine 2014; 32:725–32.

8. Donken R, Knol MJ, Bogaards JA, van der Klis FR, Meijer CJ, de Melker HE. Inconclusive evidence for non-inferior immunogenicity of two- compared with three-dose HPV immunization schedules in preadolescent girls: A systematic review and meta-analysis. J Infect 2015; 71:61–73.

9. Jit M, Choi YH, Laprise JF, Boily MC, Drolet M, Brisson M. Two-dose strategies for human papillomavirus vaccination: how well do they need to protect? Vaccine 2014; 32:3237–42.

10. Romanowski B, Schwarz TF, Ferguson L, et al. Sustained immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine administered as a two-dose schedule in adolescent girls: five-year clinical data and modeling predictions from a randomized study. Hum Vaccin Immunother 2016; 12:20–9.

11. Puthanakit T, Huang LM, Chiu CH, et al. Randomized Open Trial Comparing 2-Dose Regimens of the Human Papillomavirus 16/18 AS04-Adjuvanted Vaccine in Girls Aged 9-14 Years Versus a 3-Dose Regimen in Women Aged 15-25 Years. J Infect Dis 2016; 214:525–36.

12. Huang LM, Puthanakit T, Cheng-Hsun C, et al. Sustained immunogenicity of 2-dose human papillomavirus 16/18 AS04-adjuvanted vaccine schedules in girls aged 9-14 years: a randomized trial. J Infect Dis 2017; 215:1711–9.

13. Basu P, Bhatla N, Ngoma T, Sankaranarayanan R. Less than 3 doses of the HPV vaccine—review of efficacy against virological and disease end points. Hum Vaccin Immunother 2016; 12:1394–402.

14. D'Addario M, Redmond S, Scott P, et al. Two-dose schedules for human papillomavirus vaccine: Systematic review and meta-analysis. Vaccine 2017; 35:2892–901.

15. Naud PS, Roteli-Martins CM, De Carvalho NS, et al. Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine: final analysis of a long-term follow-up study up to 9.4 years post-vaccination. Hum Vaccin Immunother 2014; 10:2147–62.

16. Kjaer SK, Nygård M, Dillner J, et al. A 12-year follow-up on the long-term effectiveness of the quadrivalent human papillomavirus vaccine in 4 Nordic countries. Clin Infect Dis 2018; 66:339–45.

17. Nygård M, Saah A, Munk C, et al. Evaluation of the long-term anti-human papillomavirus 6 (HPV6), 11, 16, and 18 immune responses generated by the quadrivalent HPV vaccine. Clin Vaccine Immunol 2015; 22:943–8.

18. Lazcano-Ponce E, Salmerón-Castro J, García-Carrancá A, et al. Recommendations for the definition of a policy on vaccination against papillomavirus in Mexico. strategic advisory group of experts of the World Health Organization. Salud Publica Mex 2009; 51:336–41.

19. Gilca V, Sauvageau C, Boulianne N, et al. The effect of a booster dose of quadrivalent or bivalent HPV vaccine when administered to girls previously vaccinated with two doses of quadrivalent HPV vaccine. Hum Vaccin Immunother 2015; 11:732–8.

20. Hernández-Ávila M, Torres-Ibarra L, Stanley M, et al. Evaluation of the immunogenicity of the quadrivalent HPV vaccine using 2 versus 3 doses at month 21: An epidemiological surveillance mechanism for alternate vaccination schemes. Hum Vaccin Immunother 2016; 12:30–8.

21. Food and Drug Administration. Highlights of prescribing information. Cervarix [human papillomavirus bivalent (types 16, 18) vaccine, recombinant]. 2018. https://www.fda.gov/downloads/biologicsbloodvaccines/vaccines/approvedproducts/ucm186981.pdf. Accessed 16 May 2018.

22. Food and Drug Administration. Highlights of prescribing information. Gardasil [human papillomavirus quadrivalent (types 6, 11, 16, and 18) vaccine, recombinant]. 2018. https://www.fda.gov/downloads/biologicsbloodvaccines/vaccines/approvedproducts/ucm111263.pdf. Accessed 16 May 2018.

23. Bisset SL, Wilkinson D, Tettmar KI, et al. Human papillomavirus antibody reference reagents for use in postvaccination surveillance serology. Clin Vaccine Immunol 2012; 19:449–51.

24. Deschuyteneer M, Elouahabi A, Plainchamp D, et al. Molecular and structural characterization of the L1 virus-like particles that are used as vaccine antigens in Cervarix”, the AS04-adjuvanted HPV-16 and -18 cervical cancer vaccine. Hum Vaccin 2010; 6:407–19.

25. Dessy FJ, Giannini SL, Bougelet CA, et al. Correlation between direct ELISA, single epitope-based inhibition ELISA and pseudovirion-based neutralization assay for measuring anti-HPV-16 and anti-HPV-18 antibody response after vaccination with the AS04-adjuvanted HPV-16/18 cervical cancer vaccine. Hum Vaccin 2008; 4:425–34.

26. Schwarz TF, Galaj A, Spaczynski M, et al. Ten-year immune persistence and safety of the HPV-16/18 AS04-adjuvanted vaccine in females vaccinated at 15-55 years of age. Cancer Med 2017; 6:2723–31.

27. Leung TF, Liu AP, Lim FS, et al. Comparative immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and 4vHPV vaccine administered according to two- or three-dose schedules in girls aged 9-14 years: Results to month 36 from a randomized trial. Vaccine 2018; 36:98–106.
28. Toh ZQ, Russell FM, Reyburn R, et al. Sustained antibody responses 6 years following 1, 2, or 3 doses of quadrivalent human papillomavirus (HPV) vaccine in adolescent fijian girls, and subsequent responses to a single dose of bivalent HPV vaccine: a prospective cohort study. Clin Infect Dis 2017; 64:852–9.

29. Bhatla N, Nene BM, Joshi S, et al.; Indian HPV vaccine study group. Are two doses of human papillomavirus vaccine sufficient for girls aged 15-18 years? Results from a cohort study in India. Papillomavirus Res 2018; 5:163–71.

30. Einstein MH, Takacs P, Chatterjee A, et al.; HPV-010 Study Group. Comparison of long-term immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and HPV-6/11/16/18 vaccine in healthy women aged 18-45 years: end-of-study analysis of a Phase III randomized trial. Hum Vaccin Immunother 2014; 10:3435–45.

31. Scherer EM, Smith RA, Carter JJ, et al. Analysis of memory B-cell responses reveals suboptimal dosing schedule of a licensed vaccine. J Infect Dis 2018; 217:572–80.

32. Giannini SL, Hanon E, Moris P, et al. Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only. Vaccine 2006; 24:5937–49.

33. Gayet C, Gutiérrez JP. [Sexual debut in Mexico: a comparison of household national surveys]. Salud Publica Mex 2014; 56:638–47.

34. Bosch X. Eradication of cervical cancer in Latin America. Salud Publica Mex 2016; 58:97–100.

35. Safaeian M, Sampson JN, Pan Y, et al. Durability of protection afforded by fewer doses of the HPV16/18 vaccine: the CVT trial. J Natl Cancer Inst 2018; 110:1–8.

36. Sampson JN, Hildesheim A, Herrero R, Gonzalez P, Kreimer AR, Gail MH. Design and statistical considerations for studies evaluating the efficacy of a single dose of the human papillomavirus (HPV) vaccine. Contemp Clin Trials 2018; 68:35–44.

37. Kreimer AR, Herrero R, Sampson JN, et al. Evidence for single-dose protection by the bivalent HPV vaccine-review of the Costa Rica HPV vaccine trial and future research studies. Vaccine 2018; 36:4774–82.