REVIEW

Less than 3 doses of the HPV vaccine – Review of efficacy against virological and disease end points

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ABSTRACT

World Health Organization (WHO) recommended 2 doses of the Human Papillomavirus (HPV) vaccine for girls below 15 y on the basis of the immune-bridging studies demonstrating non-inferior immune response of 2 doses in the adolescent girls compared to 3 doses in the young adult women in whom the efficacy against disease is established. The biological nature of the antigens (virus-like particles) constituting the HPV vaccine is responsible for the vigorous antibody response that may make the third dose redundant. The protection offered by 2 doses has been demonstrated in non-randomized clinical trials to be comparable to that offered by 3 doses against incident and persistent infections of vaccine targeted HPV types. However, results emerging from the ecological and nested case-control studies embedded in the population based screening programs of different countries indicate reduced efficacy of 2 doses against virological and disease end points. Some recent studies observed the protective effect of single dose of the vaccine against incident and persistent infections of the vaccine targeted HPV types to be similar to 3 doses in spite of immunological inferiority. The sample size, duration of follow-ups and number of events were limited in these studies. Longer follow ups of the less than 3 doses cohorts in the ongoing studies as well as appropriately designed and ethically justifiable randomized studies are needed to establish the protection offered by the alternative schedules at least beyond 10 y of vaccination.

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Introduction

The age of the recipient and the number of doses administered differentially impact the development of Human Papillomavirus (HPV) type specific humoral immune response and the protection offered by the HPV vaccines.¹ The pivotal phase III efficacy trials of the HPV vaccines demonstrated nearly 100% efficacy of 3 doses in preventing cervical intraepithelial neoplasia 3 (CIN 3) caused by the vaccine targeted HPV types in girls and women aged between 15 to 26 y who were naïve to HPV infections.² The efficacy of 3 doses of the vaccine in girls aged 9 to 15 y was evaluated only through immunogenicity bridging trials due to the ethical and logistic impracticality of assessing disease end points at this age. The immune-bridging trials demonstrated non-inferior antibody responses in the younger girls compared to the older girls and women in whom the efficacy of the vaccine against the disease end points was established. The vaccines were initially licensed on the basis of these efficacy and immune-bridging trials for the girls and women between 9 to 26 y of age and 3 doses were recommended.

The younger girls were observed to be more immune-competent in the HPV vaccine trials and the geometric mean titres (GMTs) of the antibodies against all HPV types were nearly 2.0-fold higher among the adolescent girls than in the adult women.³ Such potent and consistently high immune response in younger girls lead to the hypothesis that less than 3 doses of

the vaccine might offer enough protection in girls aged below 15 y. A number of randomized and non-randomized studies demonstrated that the antibody response elicited by 2 doses administered at least 6 months apart in adolescent girls was non-inferior to that elicited by 3 doses of the HPV vaccine.^{4–7} The earliest evidence on the protective effect against infection of less than 3 doses of the vaccine was put forward by the post-hoc analysis of the results of some of the phase III efficacy trials of the bivalent vaccine (2vHPV) in which some of the girls/women received single or 2 doses by default.⁸ The results also provided the early but exciting indication that even single dose of the vaccine might be effective.

Starting in the year 2007 the HPV vaccines were introduced in the national immunization programs of more than 60 countries across the globe.⁹ Proof of vaccine efficacy in partially vaccinated girls/women emerged from database linkage studies or the case-control studies nested in some of these population-based programs. The results of these ecological studies demonstrated the reduced efficacy of incomplete vaccination against different disease end-points though the studies were underpowered and had selection biases. The Strategic Advisory Group of Experts (SAGE) of the World Health Organization (WHO) critically evaluated the available evidence in the year 2014 and recommended 2 doses of the HPV vaccine for the girls below 15 y of age, primarily based on the immunogenicity results.¹⁰

Some authors have, however, expressed concerns regarding the long-term protection offered by less than 3 doses of the vaccine based on the waning antibody response after a few years and weaker memory T-cell responses.¹¹ Many countries continue with the 3 doses schedule of HPV vaccine in the national immunization program.

In the present article we have discussed the biological plausibility of using less than 3 doses of the HPV vaccine and reviewed the updated evidence on the efficacy of less than 3 doses of the vaccine against virological as well as disease endpoints. The evidences from the clinical trials and the ecological studies regarding the efficacy of fewer than 3 doses of the HPV vaccine are summarized in Table 1 and are discussed in the subsequent sections.

Biological plausibility of less than 3 doses

The HPV vaccines are constituted with the L1 surface proteins of the targeted HPV types as the antigens. The antigens stimulate production of serum neutralizing anti-HPV IgG antibodies to the targeted HPV types. The neutralizing antibodies exuded at the basal stem cells of epithelial mucosa bind to the viral particles and prevent their entry inside the cells.¹² The L1 protein, when produced in an artificial system, self-assembles into the shape of an HPV virus. These non-infectious virus-like particles (VLP) have repetitive closely-spaced epitopes of the antigen protein on the surface that stimulate a vigorous immune response similar to that induced by live attenuated vaccines rather than the sub-unit vaccines.² The high immunogenicity of the vaccine is demonstrated by the high seroconversion rates even after less than 3 doses of the HPV vaccine for all targeted types. At six months after a single dose of the vaccine, 94–100% of the 9–10 y old girls had detectable antibodies in randomized controlled trials.¹³

The originally recommended schedule of the HPV vaccines was to administer 2 priming doses (at one or 2 months interval) and a single booster dose at 6 months. A single priming dose is likely to be adequate for such a vigorously immunogenic vaccine, especially in adolescent populations with high immune-competence. The memory B-cells require at least 4–6 months to mature and differentiate into high-affinity B-cells. The booster dose at 6 months can efficiently reactivate the memory B-cells to give a long lasting protection. It is therefore important to maintain the spacing between the doses of the vaccine to at least 6 months, if 2 doses are administered. Studies have documented optimal B-cell induction after 2 doses of HPV vaccine administered at an interval of 6 months in adolescent girls (9–13 years).¹

The immune correlate of protection against infection and the minimum protective antibody titers required for a vaccine schedule to be considered efficacious are still unknown for the HPV vaccines. Very low HPV antibody titers have been demonstrated to be protective in animal models.¹⁴ The antibody titres induced even by 2 doses of the vaccine are several fold higher than the levels of naturally acquired antibodies observed in women known to clear the HPV infection.¹⁵ The immune memory and the affinity and avidity of the antibodies are likely to be more important than the antibody titer in the long run. The range of HPV 16 and 18-specific memory B-cells and

CD4⁺ T-cells induced by 2 doses were demonstrated to be same as 3 doses of the vaccine.¹⁵ Studies observed comparable antibody avidity between 2- and 3-dose schedules even at 48 months after vaccination.¹⁶ Such evidences substantiate the fact that 2 doses of the HPV vaccine may be adequate in providing long lasting protection against disease from immune-biological point of view.

Even a single dose of the vaccine may suffice if the antibody induced is adequate quantitatively and qualitatively to prevent persistent infection. There is a theoretical yet unsubstantiated possibility that natural infection can serve as an immune booster and generate enough antibodies to neutralize the virus at the mucosal surface. The avidity of the type specific HPV antibodies induced after a single dose of quadrivalent (4vHPV) vaccine has been observed to be non-inferior to that induced after 3 doses even after 4 y.¹⁷ However, robust and consistent evidence demonstrating efficacy in prevention of disease is required for any change in the recommended vaccination schedule.

Efficacy of fewer than 3 doses against virological endpoints

The impact of the vaccines can be assessed by documenting the decline in the prevalence of infection from HPV types targeted by the vaccines (HPV types 16 and 18 for 2vHPV and HPV types 6, 11, 16, and 18 for 4vHPV vaccine).¹⁸

A phase III randomized controlled study was conducted by the National Cancer Institute (NCI) of USA in the Guanacaste province of Costa Rica to evaluate the safety and efficacy of the bivalent vaccine in women aged 18 to 25 y.¹⁹ An ancillary post-hoc analysis of the study evaluated the efficacy of fewer than 3 doses in the participants who received 2 doses (N = 422; 11.2% of the total participants) or a single dose (N = 196; 5.4% of the total participants) due to colposcopy referral, pregnancy, adverse events or non-compliance.⁸ The vaccine efficacies against 12 months incident persistent HPV 16/18 infection after a median follow up of 4.2 y in the women negative for HPV 16/18 DNA at baseline were 80.9% (95% CI: 71.1 – 87.7%), 84.1% (95% CI: 50.2 – 96.3) and 100% (95% CI: 66.5 – 100.0%) among the recipients of 3 doses, 2 doses and a single dose respectively. This was the first study to report the HPV vaccine efficacy against virological endpoints with a single or 2 doses of the vaccine. However, concerns were expressed due to possible selection bias and fewer participants, especially in the single dose arm.

The efficacy of 2 doses of the 2vHPV vaccine against the virological endpoint was also reported as the sub-analysis of a phase III randomized controlled study that enrolled 15 to 25 y old girls/women irrespective of their baseline cytology or HPV DNA and serology status.²⁰ Each subject in this pivotal efficacy trial was scheduled to receive 3 doses of the 2vHPV vaccine (at 0, 1 and 6 months) but 5.2% (N = 977) of the participants received 2 doses only. The efficacy of 2 doses of the vaccine in the initially HPV naïve subjects at 48 months of follow up against HPV type 16/18 incident infection was 84.5% (95% CI 31.7–98.3%) and against 6 months persistent infection was 100% (95% CI 33.1–100%).

Table 1. Summary of the evidences obtained from clinical trials and ecological studies for the efficacy of less than 3 doses of HPV vaccine.

Efficacy end point	Authors	Study setting	Vaccine used	Participant age	Key findings
Prevention of 12 months persistent infection from HPV 16/18	Kreimer et al. 2011	Sub-analysis of the Phase III efficacy trial in Costa Rica. Total 618 participants received single or 2 doses of the vaccine. Median follow up was for 4.2 years	2vHPV	18–25 years	Vaccine efficacy (VE) in the HPV 16/18 naïve participants: <ul style="list-style-type: none"> • 3-doses: 80.9% (95% CI 71.1–87.7%) • 2-doses: 84.1% (95% CI 50.2 – 96.3%) • 1-dose: 100% (95% CI 66.5 – 100%) VE of 2 doses in the HPV 16/18 naïve participants: <ul style="list-style-type: none"> • Against incident infection: 84.5% (95% CI 31.7–98.3%) • Against persistent infection: 100% (95% CI 33.1–100%) VE of 3 doses in the HPV 16/18 naïve participants; ³⁵ <ul style="list-style-type: none"> • Against persistent infection: 94.3% (96.1% CI 91.5–96.3%) VE in participants who were DNA negative for all oncogenic types, sero-negative for HPV 16/18 and had negative cytology at study entry: <ul style="list-style-type: none"> • Against incident infection: 81.4% (95% CI 78.7 – 83.8%) • Against persistent infection: 81.2% (95% CI 59.5 – 92.3%) Total 2649 women were tested for cervical HPV as they reached 18 y of age. The frequencies of incident HPV 16/18 infection were as follows: <ul style="list-style-type: none"> • 3-doses: 0.4% • 2-doses (at least 6 months interval): 0.8% • 2-doses (2 months interval): 1.3% • 1-dose: 1.1% Persistent HPV 16/18 infections were not seen in any of the groups Odds of HPV 16/18 infections in the vaccinated vs. unvaccinated groups: <ul style="list-style-type: none"> • OR for 3-doses: 0.43 (95% CI 0.31–0.45%) • OR for 2-doses: 0.61 (95% CI 0.38–0.99%) • OR for 1 dose: 0.88 (95% CI 0.48–1.6%)
Prevention of incident and 6 months persistent infection from HPV 16/18	EMA; CHMP. 2013	Sub-analysis of Phase III global efficacy (PATRICIA) Trial. Total 977 participants received 2 doses of the vaccine. Follow up was for 4 years	2vHPV	15–25 years	
Prevention of incident and 12 months persistent infection of HPV 16/18	Kreimer et al. 2015	Combined analysis of the Costa Rica vaccine trial and PATRICIA trial. Total 1185 participants received 2 doses and 543 participants received single dose. Follow up was for 4 years	2vHPV	15–25 years	
Reduction in the prevalence of HPV 16/18	Sankaranarayanan et al. 2015	Originally designed as a randomized trial in India to compare 2 versus 3 doses; the study had to suspend vaccination midway. This resulted in individuals receiving different doses of vaccine. The participants were followed up for a median 4.8 years	4vHPV	10–18 years	
Prevention of genital warts (GW)	Kavanagh et al. 2014	As part of National HPV Surveillance Program in Scotland HPV genotyping was done in residual cervical samples of women attending first cervical screening at 20 y of age. Vaccination status was obtained through data linkage. Vaccination of 13–17 y old girls commenced in 2008. An open cohort of 1,045,165 girls/women in Sweden was followed up between 2006–10 for HPV vaccination and first occurrence of GW. Mean follow up was for 3.8 years	2vHPV	20–21 years	
	Herweijer et al. 2014		4vHPV	10–24 years	Incidence rate ratios (IRR) of GW between vaccinated and unvaccinated populations aged 10–16 y at first vaccination: <ul style="list-style-type: none"> • 3-doses: 0.18 (95% CI 0.15–0.22%) • 2-doses: 0.29 (95% CI 0.21 – 0.4%) • 1-dose: 0.31 (95% CI 0.2–0.49%)

Table 1. (Continued)

Efficacy end point	Authors	Study setting	Vaccine used	Participant age	Key findings
Prevention of CIN 2+	Crowe et al. 2014	Case control study among female residents of Queensland, Australia who were eligible for catch-up vaccination and had first cervical screening during 2007–11. Women with CIN 2+ diagnosis on histology were ‘cases’ and cytology negative women were ‘controls’	4vHPV	11–27 y in 2007	Adjusted exposure odds ratios between the vaccinated and unvaccinated women were as follows: <ul style="list-style-type: none"> • 3-doses: 0.54 (95%CI 0.43–0.67%) • 2-doses: 0.79 (95%CI 0.64–0.98%) • 1-dose: 0.95 (95%CI 0.77–1.16%)
	Gertig et al. 2014	A retrospective cohort of 14,085 unvaccinated and 24,871 vaccinated women eligible for school based vaccination in 2007 and had a pap smear obtained during 2007–11 was established in Victoria, Australia through data linkage between vaccination and screening registries	4vHPV	< 17 y in 2007	Significantly lower rate of CIN 2+ disease was observed in the women receiving 3 doses compared to unvaccinated women (HR: 0.62; 95% CI 0.48–0.79) before screening. No protection was observed in women receiving single (HR: 1.26; 95% CI 0.78–2.04) or 2 doses (HR: 1.02; 95% CI 0.66–1.58).
	Brotherton et al. 2015	A retrospective cohort of 289,478 women eligible for catch-up vaccination in 2007 and had a pap smear obtained during 2007–11 was established in Victoria, Australia through data linkage between vaccination and screening registries	4vHPV	< 26 y in 2007	Significantly lower rate of CIN 2+ disease was observed in the women receiving 3 doses compared to unvaccinated women (HR: 0.86; 95% CI 0.78–0.94) before screening. No protection was observed in women receiving single (HR: 1.19; 95% CI 0.99–1.43) or 2 doses (HR: 1.21; 95% CI 1.02–1.44).

EMA; CHMP: European Medical Association; Committee for Medicinal Products for Human Use
 HR: Hazard Ratio

The results of the Costa Rica vaccine trial and the GlaxoSmithKline sponsored PATRICIA (PApilloma TRIal against Cancer In young Adults) trial, both evaluating the efficacy and safety of the 2vHPV vaccine, were analyzed together to estimate the vaccine efficacy for fewer than 3 doses with greater precision.¹⁸ The eligibility criteria, study designs and the assays used for outcome measures were similar in both the studies except the fact that the PATRICIA trial recruited 15 to 25 y old girls/women while the participants of the Costa Rica trial were between 18 to 25 y of age. In both trials there were girls/women who did not complete the 3 doses schedule of the vaccine. The analysis was done in the modified total vaccinated cohort that excluded the women who were HPV DNA positive for the type under consideration at enrollment. The cohort included 1185 women receiving 2 doses and 543 women receiving one dose. After 4 y of follow up the rates of one time detection of incident HPV 16/18 infection were similar in the women receiving single dose, 2 doses and 3 doses of the vaccine. The vaccine efficacies against incident HPV 16/18 infection for a single dose, 2 doses and 3 doses were 87.5% (95% CI 60.9–97.1%), 81.2% (95% CI 59.5–92.3%) and 81.4% (95% CI 78.7–83.8%) respectively. The study also evaluated the vaccine efficacy against HPV 16/18 infection persistent beyond 6 months and 12 months as secondary endpoints. Single or 2 doses of the vaccine were as efficacious as 3 doses in preventing persistent HPV 16/18 infection. The efficacy of different doses of the vaccine to prevent incident and persistent HPV 31/33/45 infections (cross-protection) was also assessed. The cross-protective efficacy against incident infection was demonstrated for 2 doses (37.7%; 95% CI 12.4 to 55.9), though the efficacy was inferior to that of 3 doses (59.7%; 95% CI 56.0 to 63.0). No cross-protective effect of 2 doses was observed if the interval between the doses was less than 6 months. Some cross-protective efficacy of a single dose against incident infection or of single and 2 doses against 6 months and 12 months persistent infection were also demonstrated, but none of them were statistically significant as the number of events was very few.

A cluster randomized clinical trial was initiated in the year 2009 at multiple sites in India to compare 3 doses of the 4vHPV vaccine to 2 doses (administered at 0 and 6 months) to girls/women aged 10 to 18 y.¹⁷ The vaccination was suspended by the Government of India due to reasons unrelated to the study. The suspension resulted in the formation of cohorts of girls (N = 17,729) receiving different doses of the vaccine at different time points (3 doses at 0, 2 and 6+ months, 2 doses at 0 and 2 months, 2 doses at 0 and 6+ months and single dose) by default. The geometric mean titres (GMT) of neutralizing antibodies at 18 months after the first dose for HPV types 6/11/16 in the 2 dose group were non-inferior to that of the 3-dose group. The GMT for HPV 18 was inferior in the 2-dose group compared to the 3-dose group. The GMTs for HPV 6/11/16/18 in girls/women receiving 2 doses at 2 mo interval or single dose were inferior to those receiving 3 doses. However, the quality and binding affinity of the antibodies as estimated by the geometric mean avidity index for all 4 HPV types in the alternate dosage schedules were the same as the 3 dose schedule. The frequencies of the incident and the persistent infections with vaccine targeted HPV as well as non-targeted high-risk HPV types (cross-protection) were estimated based on the analysis of the

samples collected from 2649 vaccinated women after they reached 18 y of age. After a median follow up time of 4 to 6 y the frequencies of HPV 16/18 incident infection were 0.4% (95%CI 0.0–1.3) in women receiving 3 doses; 0.8% (0.2–1.9) in women receiving 2 doses at the interval of 6 months or more; 1.3% (0.6–2.4) in women receiving 2 doses at 2 months interval and 1.1% (0.6–2.1) in women receiving a single dose. The frequencies of incident infection were higher in the groups receiving alternate schedules compared to 3 doses groups, though the differences were not statistically significant. No persistent HPV 16 and 18 infections were seen in any of the 838 vaccinated women for whom 2 or more samples were available for analysis during the median follow up of 4.7 y.

The efficacy of fewer doses of the HPV vaccine against virological endpoints from the national immunization programs was reported from Scotland.²¹ A school-based program to vaccinate the 12 to 13 y old girls using the 2vHPV vaccine was introduced in Scotland in the year 2008. Catch up vaccination was offered to 13 to 17 y old girls. As part of a nationwide HPV surveillance program residual cervical samples were collected for HPV genotyping from the women attending cervical cancer screening for the first time at the age of 20 y. Between 2009 and 2012 residual liquid based cytology samples were collected from 1000 women every year. The HPV vaccination status of these women was obtained from the national immunization register. A total of 4729 samples were collected; out of them 23.4% were fully vaccinated and 3.4% were partially vaccinated. The fully vaccinated women had significantly lower prevalence of HPV 16 and 18 compared to the unvaccinated women (13.6% vs 29.8%; OR 0.43 95%CI 0.31 – 0.45). Women receiving 2 doses also had lower prevalence of the vaccine targeted HPV types (20.8%; OR 0.61, 95%CI 0.38–0.99). Women receiving a single dose did not have any significant reduction in HPV type 16 and 18 prevalence (27.3%; OR 0.88, 95%CI 0.48–1.6) compared to the vaccinated women. Evidence of cross protection against types 31, 33 and 45 was observed in fully vaccinated women (OR 0.53; 95%CI 0.38 – 0.74) and also in women receiving 2 doses (OR 0.55; 95%CI 0.26 – 1.17). No cross-protective effect was observed for a single dose of the vaccine.

Efficacy of less than 3 doses to prevent Genital warts

Genital warts have a short incubation period of 1 to 6 months and a much shorter natural history than that of cervical neoplasia.²² The earliest evidence of the effectiveness of the 4vHPV vaccination at the population level is indicated by the reduction in the incidence of genital warts. Such evidence was obtained from ecological studies in the countries that introduced 4vHPV vaccines in the national immunization programs.^{23,24} Some of these studies also reported the efficacy of fewer than 3 doses of the 4vHPV vaccine in prevention of genital warts.

Australia was the first country to introduce the 4vHPV vaccine in the national immunization program in April 2007.²⁵ The primary aim of the program was to vaccinate the girls between 12 to 13 y of age. Girls and women in the 13 to 26 y age group were also vaccinated through the catch-up program between 2007 and 2009. A sentinel surveillance network monitored the effect of vaccination on the burden of genital warts reported in the sexual health services across the country. The

initial reports published in 2011 documented a 59% reduction in the diagnosis of genital warts in girls/women who were 12 to 26 y old in the year 2007.²³ Heterosexual men of the same age also had 39% reduction in genital wart diagnosis due to herd immunity. In a subsequent update of the study it was noted that no genital warts were diagnosed in women below 21 y of age who reported to be vaccinated.²⁶ This was in spite of the fact that some of these women received fewer than 3 doses of the vaccine, although the number was likely to be low due to the high coverage of 4vHPV vaccine in Australia.

HPV vaccination was introduced in Sweden in 2007 and girls between 13 to 17 y were vaccinated with the 4vHPV vaccine.²⁷ An open cohort of 1,045,165 girls and young women aged 10 to 24 y were followed up for a mean duration of 3.8 y. Two doses of the vaccine were associated with significant reduction in the risk of genital warts especially in women receiving the first dose of the vaccine below the age of 17 y. The incidence rate ratio (IRR) for genital warts among the recipients of 3 doses below 17 y of age was 0.18 (95% CI, 0.15–0.22) compared to unvaccinated women. Two doses were associated with an IRR of 0.29 (95% CI, 0.21–0.40), and even a single dose was associated with an IRR of 0.31 (95% CI, 0.20–0.49). In the study population 3 doses of the vaccine could prevent 59 additional cases of condyloma per 100,000 person years compared to 2 doses, a difference that the authors considered to be small. No herd immunity was observed in the unvaccinated women possibly because of the low overall vaccine uptake rate (25%) in the population.

Efficacy of fewer than 3 doses to prevent CIN or worse disease

The efficacy of full and partial courses of the HPV vaccine was estimated among the female residents of Queensland, Australia who were eligible for the catch-up vaccination program (born between July 1980 and July 1997) and had their first cervical cancer screening test during the study period between 1 April 2007 and 31 March 2011.²⁸ In the case-control study the women with histologically confirmed high grade lesions (cervical intraepithelial neoplasia 2 or worse; CIN 2+) registered with the Queensland pap smear registry were considered as 'cases' (N = 1062). Women who had any abnormal cytology result but did not have histology proved CIN 2+ lesions were included as 'other cases' (N = 10887). All cytology negative women were included in the controls (N = 96404). The study observed 46% protection against high-grade lesions in the recipients of 3 doses of the vaccine (adjusted OR 0.54; 95%CI 0.43–0.67). The protection offered by 2 doses of the vaccine was 21% (adjusted OR 0.79; 95%CI 0.64–0.98). No significant protection was observed for single dose of the vaccine.

A database linkage study (between the cervical cytology registry and the HPV vaccination program register) in Victoria Province of Australia identified a cohort of women who were 17 y or younger in 2007 (so eligible for school based vaccination) and had a Pap smear recorded during the study period between 1 April 2007 and 31 December 2011.²⁹ In the cohort there were 14,085 unvaccinated women, 1,422 women who received one dose, 2,268 women who received 2 doses and 21,151 women who received all 3 doses of the vaccine. Since

the cervical neoplasias detected during the course of vaccination are due to prevalent HPV infections, the vaccine efficacy assessment was performed after excluding the person-time for those in which the disease was detected between dose 1 and dose 3. The study observed significantly lower rates of histology proved high grade abnormalities (CIN 2, CIN 3, adenocarcinoma in situ) in the completely vaccinated population (hazard ratio 0.62; 95% C.I. 0.48–0.79) compared to the unvaccinated one. No protection against high grade diseases was observed in both single (hazard ratio 1.26; 95% C.I. 0.78–2.04) and 2 doses (hazard ratio 1.02; 95% C.I. 0.66–1.58) groups.

Brotherton et al. identified another cohort of women in Victoria Province of Australia using the same database linkages used in the earlier study. The women in the cohort were 26 y or younger in 2007 (so eligible for primary or catch-up 4vHPV vaccination) and had a Pap smear recorded during the study period between 1 April 2007 and 31 December 2011.³⁰ They observed protection against both low and high grade cytological abnormalities from any number of doses of the vaccine so long as the vaccines were administered prior to onset of screening. Women vaccinated after onset of screening were sexually active and had high risk of harbouring HPV infection at the time of vaccination. The protective effects against high grade cytological abnormalities of single dose (HR 0.44; 95% CI 0.32–0.59), 2 doses (HR 0.63; 95% CI 0.50–0.80) and 3 doses (HR 0.53; 95% CI 0.47–0.60) were consistent across the age groups. Consistent with the observations of earlier cohort analysis they also did not observe any protective effect of fewer doses of the vaccines against histologically proved high grade lesions. However, a subset analysis of the youngest group of participants (<16 years during vaccination) did demonstrate protection (though not statistically significant due to low sample size) both for the single dose group (HR 0.83; 95% CI 0.33 – 2.05) and the 2-dose group (HR 0.70; 95% CI 0.32–1.53).

The Australian studies in Queensland and Victoria had selection biases, since the groups receiving fewer than 3 doses were of older age at the time of vaccination and had first screening at younger age signifying early sexual debut and higher risk of being HPV infected at the time of vaccination. In fact, the second study in Victoria observed that the effect of partial vaccination became more evident with time (after 48 months) as the prevalent lesions in the women already HPV infected at the time of vaccination were detected and treated or cleared and the incident lesions continued to occur in the unvaccinated women but not the women receiving at least a single dose.³⁰ Smaller numbers of women in the single or 2 dose groups was responsible for less precision of the estimates.

Discussion

The accumulated evidences in favor of 2 doses of the vaccine administered at >6 months interval encouraged Brazil, Chile, Germany, Netherlands, Quebec, South-Africa, Switzerland and the United Kingdom to adopt a 2-dose schedule for the national immunization program. The developing countries are required to implement the 2-dose vaccination schedule to get support from the GAVI Alliance. However, the results of some of the studies indicate that the quantum of protection offered by 2 doses may not be as high as that offered by 3 doses against

genital warts or cervical neoplasias. Skepticism has been expressed regarding the durability of protection based on some of the studies that showed waning of the antibody levels after 2 doses during follow-up beyond 2 y and loss of sero-positivity to HPV 18 in nearly 40% of the vaccinated girls.¹¹ Majority of the countries in the developed world retain the 3 doses schedule. It can be argued that there is still no evidence to indicate that the reduced titer of antibody or sero-negativity to a particular HPV type is associated with breakthrough infection or disease after vaccination.³¹ Even then, robust evidence is required from long term follow up of the young girls vaccinated with 2 doses to conclusively establish the current WHO policy primarily based on immunological non-inferiority of 2 doses. As the immune correlate of protection against HPV infection is unknown, the criteria selected for non-inferiority margin are arbitrary and debatable.¹¹ Demonstration of protection against at least 6 months persistent infection will be more appropriate for the alternative dosing schedules to be widely accepted rather than simple demonstration of immunological non-inferiority.³²

The major limitation of the studies that evaluated the efficacy of fewer than 3 doses of the vaccine against virological end points is that none of them are appropriately designed randomized controlled trials. The evidence of protective effects of single or 2 doses of the vaccine against incident or persistent infection have been obtained by default through the follow-up of the incompletely vaccinated participants of the major efficacy trials. Though the studies have showed that 2 doses spaced at 6 months offer definite protection and that even a single dose has protective effect, acceptance of the results is somewhat guarded due to less number of subjects in the defaulter groups and/or inadequate follow-up and possible selection bias. The numbers of events (incident and persistent infections) in these studies were too little due to the small number of participants and limited follow-ups.

The ecological studies nested into the national immunization programs in Australia and Sweden have produced evidence of efficacies of the incomplete doses of the 4vHPV vaccine (albeit lower than 3 doses) in preventing genital warts. The evidence to support the efficacy of the less number of doses to prevent cervical neoplasia will take some more time to emerge, given the long natural history of the disease and the young age of the vaccinated girls. The real efficacies of the different dosage schedules of the vaccine will be more evident as the youngest cohorts of girls receiving vaccine before sexual debut reach the age of screening after few more years. The only studies reporting efficacies of single or 2 doses in prevention of high grade disease (on cytology or histology) from participants of the national immunization program are from Australia.^{28,29,30} As discussed earlier, the studies suffered from selection biases that could diminish the efficacy of fewer doses. The participants of these studies were older at the time of vaccination compared to the target age recommended for 2 doses of the vaccine. Even then, the inferior protection observed with less than 3 doses against the most valid histology proved CIN endpoint is a matter of concern. The observed efficacies could even be inflated due to herd immunity skewing the results in favor of less number of doses.

As population-based vaccination becomes more widespread, the effectiveness of different dosing schedules to prevent disease

outcomes can be established by close monitoring of the cohorts of vaccinated girls. Longer follow-up of the cohorts of girls/women who received single or 2 doses by default in the ongoing study in India will provide an excellent opportunity not only to provide more robust evidence in favor of prevention of infection but also establish efficacy against high grade CIN lesions. However, such evidence will take at least another 5 y to emerge.

Some of the Latin American countries (Mexico, Colombia) have recently adopted an extended schedule of 0, 6 and 60 months to vaccinate adolescent girls in the national immunization program.⁷ The relevance of the third dose can be assessed after 5 y based on the accumulated evidence by that time. If at all needed, the delayed administration of the third dose will have a booster effect that may possibly last longer. An alternative 2 doses schedule of 0 and 12 months has non-inferior immune response to the standard 3 doses schedule in 11 to 13 y old girls.³³ Vaccinating the adolescent girls at one year interval may be logistically more convenient from programmatic point of view. However, this schedule also needs further evaluation.

The evidence to date strongly justifies the initiation of randomized controlled studies to evaluate the efficacy of alternate dosage schedules against virological or even disease end points. As the peak incidence for HPV infection is at 20 to 25 y of age, protection against infection provided by a 2-dose or single dose vaccination strategy among young girls should be demonstrated to last for at least 10 y.³⁴

Abbreviations

CIN	Cervical intraepithelial neoplasia
GMT	Geometric mean titres
HPV	Human Papillomavirus
HR	Hazard ratio
IRR	Incidence rate ratio
NCI	National Cancer Institute
SAGE	The Strategic Advisory Group of Experts
VE	Vaccine efficacy
VLP	Virus-like particles
WHO	World Health Organization
PATRICIA	PApilloma TRIal against Cancer In young Adults
OR	Odds Ratio
CI	Confidence Interval

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No potential conflicts of interest were disclosed.

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