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Two-dose recommendation for Human Papillomavirus vaccine can be extended up to 18 years – updated evidence from Indian follow-up cohort study

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ABSTRACT

Earlier publication from the ongoing multi-centric study of the International Agency for Research on Cancer to evaluate less than three doses of the quadrivalent Human Papillomavirus (HPV) vaccine in India amongst unmarried girls demonstrated non-inferior total antibody titres, neutralizing antibody titres and antibody avidity in 2-dose recipients compared to 3-dose recipients at 15–18 years of age (Bhatla et al., 2018) [7].

The number of participants recruited at 15–18 years of age was 1515 and 1795 in the 3-dose and the 2-dose groups respectively. At a median follow-up of 7 years, incident HPV 16/18 infections were detected in 1.6% women receiving two doses and 0.8% women receiving three doses at 15–18 years. Frequency of incident infection was 7.0% in the age- and site-matched unvaccinated women (N = 1484). No persistent infection from HPV 16 was observed in the 2- or 3-dose recipients and one (0.2%) persistent HPV 18 infection was documented,

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each in the 3-dose and 2-dose cohorts. Among the unvaccinated women, the frequency of HPV 16/18 persistent infection was 1.7%.

The protection offered by two doses of quadrivalent HPV vaccine against incident and persistent infections in recipients at 15–18 years is comparable to that seen in 3-dose recipients at 15–18 years.

1. Introduction

The Strategic Advisory Group of Experts (SAGE) of the World Health Organization (WHO) recommended a 2-dose schedule of the Human Papillomavirus (HPV) vaccine for girls below 15 years of age in the year 2014 [1]. The guiding principle for the WHO SAGE to recommend two doses of the vaccine for young adolescents was the International Agency for Research on Cancer (IARC)/United States National Cancer Institute (NCI) Expert Group (2013) recommendation that immunological bridging was a valid approach to determine the efficacy of fewer than three doses of HPV vaccine and was not inherently age-specific [2]. Several immunological bridging studies and their systematic reviews conclusively demonstrated that the antibody response following two doses (administered at an interval of at least six months) of the HPV vaccine in the girls below 15 years of age was non-inferior to that in older women receiving standard three doses of the vaccine, the efficacy of three doses having been already established in the second group [3,4]. The simplified vaccination schedule and the lower programmatic cost associated with 2-dose accelerated the introduction of the HPV vaccine and by January 2018, 79 countries introduced the vaccine and another 10 low- and middle-income countries (LMICs) were ready to introduce the vaccine in 2018–2019 [5].

The WHO Position Paper on HPV Vaccines (2017) recognized that targeting multiple age cohorts of girls between 9 and 18 years at the time of HPV vaccine introduction would provide significant direct protection and herd immunity, resulting in faster and greater population impact [6]. However, the recommended 3-dose schedule for girls between 15 and 18 years is a major limitation to include this age group and is less likely to be cost-effective. In our earlier publication based on the ongoing study from India we reported that the immunogenicity in 15–18 year old recipients of two doses of the quadrivalent HPV vaccine was non-inferior to that in the 15–18 years old recipients of three doses (standard of care) for all the vaccine targeted types [7]. In the present manuscript we report the comparative protection offered against incident and persistent HPV infections in recipients of two and three doses of the quadrivalent vaccine at 15–18 years of age.

2. Method

The Indian multi-centric study, originally planned as a randomized clinical trial (RCT) to compare the efficacy of two doses of the quadrivalent HPV vaccine to that of three doses, essentially became a prospective non-randomized cohort study due to the suspension of vaccination in all HPV vaccine trials by the Government of India due to reasons unrelated to the study. The methodology of this study has been described in detail elsewhere [8]. Recruitment of unmarried girls between 10 and 18 years of age to the two randomization groups was initiated in September 2009. At the time of study suspension in April 2010, 17,729 eligible girls (88.6% of the target 20,000 girls) were already vaccinated. The suspension resulted in participants receiving three doses (days 1, 60 and ≥180) two doses (days 1 and ≥180) two doses by default (days 1 and 60) and a single dose by default. Blood samples were collected from the vaccinated girls at baseline, one month after the last dose of the vaccine in 2-dose and 3-dose groups, at 12 months post-vaccination in the single dose group and yearly thereafter from a sub-set from each group for five consecutive years for immunogenicity assessment. The immunogenicity assessment included the measurement of L1 genotype-specific binding antibody titres, geometric mean neutralization titres (GMT) of targeted HPV antibodies and the antibody avidity for the vaccine targeted HPV types. The findings for the immunogenicity outcomes have been published elsewhere [7,9].

All the vaccinated girls were followed up yearly to document the occurrence of any vaccine related adverse events or new medical conditions. Cervical specimens for HPV genotyping were collected from the participants starting at 18 months after marriage or 6 months after first child-birth, whichever was earlier. The aim was to collect four specimens from each eligible woman at an interval of 12 months between two consecutive collections. A fifth sample was only collected from women being detected of an HPV infection for the first time in the fourth sample. The samples were tested by the HPV type-specific E7 polymerase chain reaction (PCR) bead-based multiplex genotyping to detect the 19 high-risk or probable high-risk types (type 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68a, 68b, 70, 73 and 82) and two low risk types (types 6 and 11). Both the genotyping and immunogenicity assays were done at the laboratory at Rajiv Gandhi Centre for Biotechnology (RGCB), India in a blinded manner. Appropriate internal and external quality control measures for the assays were performed.

A cohort of around 1500 unvaccinated married women matched for study site and age against the vaccinated participants was recruited between May 2012 and June 2015 as controls. Cervical specimens for HPV genotyping were collected from them yearly for four consecutive years.

The vaccinated married participants and the unvaccinated controls underwent cervical cancer screening once they reached 25 years of age. Digene Hybrid Capture 2™ (HC 2) high risk HPV detection test (Qiagen; Gaithersburg, USA) was used as the screening test. The HC 2 testing for cervical cell samples from all participating centres was carried out at a centralised laboratory in Nargis Dutt Memorial Cancer Hospital, Barshi, India. The HC 2 test detects the presence of 13 most common oncogenic HPV types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) in the specimen. A positive test indicated that the Relative Light Unit (RLU) reading obtained for the specimen was equal to or greater than the mean of the RLU values of the positive controls (PC) supplied along with the HC 2 kit (RLU/PC cut-off ratio ≥1.0). A positive test implied the presence of at least 5000 copies of DNA of any of the 13 oncogenic HPV types. All HC 2 positive women were recalled for colposcopy and cervical punch biopsies were obtained if an abnormality was detected on colposcopy. Genotype-specific HC 2 test to detect the presence of HPV types 16, 18 and 45 was also performed on each HC 2 positive specimen, though the results did not change the follow-up protocol. The final diagnosis among women with disease confirmation was based on histology reports or on colposcopy for those without histology reports. Each histopathology slide was reviewed independently by two pathologists at the study site. According to the current protocol, the opinion of an expert pathologist will be sought only if there is a disagreement in the diagnoses of the site pathologists. Till now no such situation was encountered. The next round of screening for the HC 2 negative women has been planned after 5 years.

The recruitment of a second unvaccinated control cohort of married women aged between 25 and 28 years (age-matched for the vaccinated married women undergoing screening) was initiated in the year 2017. They are being screened for cervical cancer with HC 2 using the same protocol as that for the vaccinated married women eligible for screening. The target is to recruit total 3500 women within two years in the second control cohort. The two control cohorts together will provide a total of 5000 unvaccinated women to be assessed for the cervical neoplasia end-point.

2.1. Definition of the outcomes

The following outcomes are being assessed in the present analysis: incidence of one-time type-specific HPV infection defined as the detection of a particular HPV type at any time during the follow-up; type-specific persistent HPV infection defined as the detection of the same type of an HPV infection at two consecutive time points at least 10 months apart with no intervening HPV negative finding of the same type in between the two positive samples; HC 2 screening test overall positivity and type-specific HPV positivity; and cervical intraepithelial neoplasia (CIN) incidence which is assessed among the HC 2 positive women who undergo colposcopy and/or biopsy for disease confirmation.

2.2. Statistical analysis

This analysis included age-stratified data of the recipients of two doses (Days 1 and ≥180) and three doses (Days 1, 60 and ≥180) The 2-dose recipients at 15–18 years of age were compared to the standard of care groups, i.e. 3-dose recipients at 15–18 years and 2-dose recipients at 10–14 years and also with the age-matched unvaccinated cohort. The outcomes were presented as a proportion with their exact binomial 95% confidence intervals (CIs). P values for the comparison of the proportions were calculated with a two-sided test on the equality of proportions using large sample statistics, which also gives exact P values.

The study has been approved by the research ethics committees of IARC and all the participating sites. The study is registered with ISRCTN

Table 1
Analysis of one-time incident HPV infections by age.

Type of HPV infection/Dose received	HPV incidence				
	Participants with ≥ one samples tested				
	Age group	Women assessed n	Women with incident infections n	Proportion of incident infections (%, 95% CI)	
HPV 16/18 infections					
3-dose (Days 1, 60, ≥180)	10–14	617	10	1.6	(0.8–3.0)
	15–18	860	7	0.8	(0.3–1.7)
2-dose (Days 1, ≥180)	10–14	611	3	0.5	(0.1–1.4)
	15–18	901	14	1.6	(0.9–2.6)
Unvaccinated group	18–23	1484	104	7.0	(5.8–8.4)
HPV 16 infections					
3-dose (Days 1, 60, ≥180)	10–14	617	10	1.6	(0.8–3.0)
	15–18	860	5	0.6	(0.2–1.4)
2-dose (Days 1, ≥180)	10–14	611	2	0.3	(0.0–1.2)
	15–18	901	7	0.8	(0.3–1.6)
Unvaccinated group	18–23	1484	68	4.6	(3.6–5.8)
HPV 18 infections					
3-dose (Days 1, 60, ≥180)	10–14	617	1	0.2	(0.0–0.9)
	15–18	860	2	0.2	(0.0–0.8)
2-dose (Days 1, ≥180)	10–14	611	1	0.2	(0.0–0.9)
	15–18	901	7	0.8	(0.3–1.6)
Unvaccinated group	18–23	1484	43	2.9	(2.1–3.9)
HPV 6/11 infections					
3-dose (Days 1, 60, ≥180)	10–14	617	7	1.1	(0.5–2.3)
	15–18	860	7	0.8	(0.3–1.7)
2-dose (Days 1, ≥180)	10–14	611	1	0.2	(0.0–0.9)
	15–18	901	8	0.9	(0.4–1.7)
Unvaccinated group	18–23	1484	46	3.1	(2.3–4.1)
Vaccine-targeted HPV (16/18/6/11) infections					
3-dose (Days 1, 60, ≥180)	10–14	617	17	2.8	(1.6–4.4)
	15–18	860	13	1.5	(0.8–2.6)
2-dose (Days 1, ≥180)	10–14	611	4	0.7	(0.2–1.7)
	15–18	901	21	2.3	(1.4–3.5)
Unvaccinated group	18–23	1484	145	9.8	(8.3–11.4)
Non-vaccine-targeted HPV 31, 33 and 45 infections					
3-dose (Days 1, 60, ≥180)	10–14	617	19	3.1	(1.9–4.8)
	15–18	860	49	5.7	(4.2–7.5)
2-dose (Days 1, ≥180)	10–14	611	23	3.8	(2.4–5.6)
	15–18	901	41	4.6	(3.3–6.1)
Unvaccinated group	18–23	1484	118	8.0	(6.6–9.4)
Non-vaccine-targeted HPV infections excluding 31, 33 and 45					
3-dose (Days 1, 60, ≥180)	10–14	617	98	15.9	(13.1–19.0)
	15–18	860	124	14.4	(12.1–16.9)
2-dose (Days 1, ≥180)	10–14	611	88	14.4	(11.7–17.4)
	15–18	901	127	14.1	(11.9–16.5)
Unvaccinated group	18–23	1484	283	19.1	(17.1–21.2)
Any HPV (16/18/6/11/26/31/33/35/39/45/ 51/52/53/56/58/59/66/68/70/73/82) infection					
3-dose (Days 1, 60, ≥180)	10–14	617	120	19.4	(16.4–22.8)
	15–18	860	158	18.4	(15.8–21.1)
2-dose (Days 1, ≥180)	10–14	611	101	16.5	(13.7–19.7)
	15–18	901	160	17.8	(15.3–20.4)
Unvaccinated group	18–23	1484	412	27.8	(25.5–30.1)

HPV: human papilloma virus; CI: confidence interval.

registry (registration number ISRCTN98283094) and ClinicalTrials.gov (registration number NCT00923702).

3. Results

There were 2833 and 1515 participants receiving three doses of the vaccine at 10–14 and at 15–18 years of age respectively. These numbers for the 2-dose recipients were 3184 and 1795 respectively. The distribution of the site and other demographic characteristics were similar in the different age and doses received group combinations as reported in our earlier publications [7,8]. The present analysis is based on updated data till 1st September 2018. The median time from first dose of vaccination to last cervical sample collection was 7.1 years (interquartile range (IQR): 6.3–7.7) in the 2-dose group and 7.1 years (IQR: 6.3–7.8) in the 3-dose group. The median time from date of marriage to date of last cervical sample collection was 3.0 years (IQR: 2.2–3.9) in the 2-dose group, 3.0 years (IQR: 2.3–4.0) in the 3-dose group and 4.7 years (IQR: 3.3–6.0) in the unvaccinated women.

For the assessment of the one-time incident HPV infections outcome, the total numbers of women evaluated in the 3-dose, age 15–18 years, and the 2-dose, ages 10–14 years and 15–18 years were 860, 611 and 901 respectively (Table 1). The unvaccinated cohort included 1484 women for this outcome. Incident HPV 16 and/or HPV 18 infections were detected in 1.6% (95% CI: 0.9–2.6%) women receiving two doses at 15–18 years of age, while this proportion in their counterparts who received 3 doses was 0.8% (95% CI: 0.3 – 1.7%). In women who

Table 2
Analysis of one-time persistent HPV infections by age.

Type of HPV infection/Dose received	HPV persistence				
	Participants with ≥ two samples tested				
	Age group	Women assessed n	Women with persistent infections n	Proportion of persistent infections (%, 95% CI)	
HPV 16/18 infections					
3-dose (Days 1, 60, ≥180)	10–14	298	0	0.0	(0.0–**)
	15–18	600	1	0.2	(0.0–0.9)
2-dose (Days 1, ≥180)	10–14	277	0	0.0	(0.0–**)
	15–18	598	1	0.2	(0.0–0.9)
Unvaccinated group	18–23	1228	21	1.7	(1.1–2.6)
HPV 16 infections					
3-dose (Days 1, 60, ≥180)	10–14	298	0	0.0	(0.0–**)
	15–18	600	0	0.0	(0.0–**)
2-dose (Days 1, ≥180)	10–14	277	0	0.0	(0.0–**)
	15–18	598	0	0.0	(0.0–**)
Unvaccinated group	18–23	1228	15	1.2	(0.7–2.0)
HPV 18 infections					
3-dose (Days 1, 60, ≥180)	10–14	298	0	0.0	(0.0–**)
	15–18	600	1	0.2	(0.0–0.9)
2-dose (Days 1, ≥180)	10–14	277	0	0.0	(0.0–**)
	15–18	598	1	0.2	(0.0–0.9)
Unvaccinated group	18–23	1228	7	0.6	(0.2–1.2)
HPV 6/11 infections					
3-dose (Days 1, 60, ≥180)	10–14	298	0	0.0	(0.0–**)
	15–18	600	1	0.2	(0.0–0.9)
2-dose (Days 1, ≥180)	10–14	277	0	0.0	(0.0–**)
	15–18	598	0	0.0	(0.0–**)
Unvaccinated group	18–23	1228	1	0.1	(0.0–0.5)
Vaccine-targeted HPV (16/18/6/11) infections					
3-dose (Days 1, 60, ≥180)	10–14	298	0	0.0	(0.0–**)
	15–18	600	2	0.3	(0.0–1.2)
2-dose (Days 1, ≥180)	10–14	277	0	0.0	(0.0–**)
	15–18	598	1	0.2	(0.0–0.9)
Unvaccinated group	18–23	1228	22	1.8	(1.1–2.7)
Non-vaccine-targeted HPV 31, 33 and 45 infections					
3-dose (Days 1, 60, ≥180)	10–14	298	1	0.3	(0.0–1.9)
	15–18	600	2	0.3	(0.0–1.2)
2-dose (Days 1, ≥180)	10–14	277	1	0.4	(0.0–2.0)
	15–18	598	2	0.3	(0.0–1.2)
Unvaccinated group	18–23	1228	10	0.8	(0.4–1.5)
Non-vaccine-targeted HPV infections excluding 31, 33 and 45					
3-dose (Days 1, 60, ≥180)	10–14	298	8	2.7	(1.2–5.2)
	15–18	600	17	2.8	(1.7–4.5)
2-dose (Days 1, ≥180)	10–14	277	11	4.0	(2.0–7.0)
	15–18	598	12	2.0	(1.0–3.5)
Unvaccinated group	18–23	1228	44	3.6	(2.6–4.8)
Any HPV (16/18/6/11/26/31/33/35/39/45/51/52/53/56/58/59/66/68/70/73/82) infection					
3-dose (Days 1, 60, ≥180)	10–14	298	9	3.0	(1.4–5.7)
	15–18	600	19	3.2	(1.9–4.9)
2-dose (Days 1, ≥180)	10–14	277	12	4.3	(2.3–7.4)
	15–18	598	15	2.5	(1.4–4.1)
Unvaccinated group	18–23	1228	68	5.5	(4.3–7.0)

HPV: human papilloma virus; CI: confidence interval.

received two doses at a younger age of 10–14 years, the proportion was 0.5% (95% CI: 0.1 – 1.4%). The proportion of unvaccinated women having incident HPV 16 and/or HPV 18 infections (7.0%; 95% CI: 5.8 – 8.4%) was significantly higher compared to those in any of the vaccinated groups (p-values < 0.001).

The proportion of women having incident infections of the HPV types 31/33/45 (cross-protected types) was 4.6% (95% CI: 3.3 – 6.1%) in the 2-dose recipients at 15–18 years, 5.7% (95% CI: 4.2 – 7.5%) in the 3-dose recipients at 15–18 years and 3.8% (95% CI: 2.4 – 5.6%) in the 2-dose recipients at 10–14 years; no significant difference being observed between the dose and age groups. (Table 1) The frequency of HPV 31/33/45 incident infections (8.0%; 95% CI: 6.6–9.4%) in the unvaccinated women was higher compared to those of the vaccinated women (all p-values < 0.05).

The proportion of women detected to have non-vaccine targeted HPV infections excluding HPV 31/33/45 was similar across the dose and age groups, suggesting similar exposure to HPV infections across groups (Table 1).

Persistence of HPV infection could be assessed in 600 women aged 15–18 years receiving three doses, 598 in women aged 15–18 years and 277 women aged 10–14 years receiving two doses, and in 1228 unvaccinated controls (Table 2). The frequency of persistent infections from the vaccine targeted HPV types was extremely low in the vaccinated girls. No persistent infection from HPV 16 was observed in the two- or three-dose recipients and one (0.2%; 95% CI: 0.0–0.9%) case of persistent HPV 18 infection was documented in the 3-dose, age 15–18 and one (0.2%; 95% CI: 0.0–0.9%) in the 2-dose, age 15–18 cohorts. Among the unvaccinated women assessed, 21 (1.7%; 95% CI: 1.1–2.6%) had persistent HPV 16/18 infections.

Persistent infections from HPV 31/33/45 were observed in two (0.3%; 95% CI: 0.0–1.2%), two (0.3%; 95% CI: 0.0–1.2%) and one (0.4%; 95% CI: 0.0–2.0%) women receiving three vaccine doses at age 15–18 years, two doses at 15–18 years and two doses at 10–14 years respectively. This figure in unvaccinated women was 10 (0.8%; 95% CI: 0.4–1.5%).

A blinded re-testing of 92 randomly selected samples from the RGCB laboratory was performed at the IARC HPV detection laboratory using the multiplex genotyping assay for quality control. HPV type specific agreement between RGCB and IARC labs ranged from 94.6% for HPV 52 to 100.0% for HPV 31, 35, 53, 66 and 70. The 2017 Global HPV DNA proficiency panel obtained from International HPV Reference Centre, Karolinska Institute, Sweden was analysed in a blinded manner at RGCB laboratory as an additional external quality control exercise. The panel included 50 International Units (IU)/ 5 µl of HPV 16 and HPV 18 DNA, and 500 genome equivalents/ 5 µl of the other HPV types. The type-specific agreement ranged from 97.7% for HPV 51 to 100% for all the other types (HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59 and 66b). The specificity of the reported types exceeded 97% and the RGCB laboratory was considered as 'proficient' based on the overall performance.

The outcomes of the screening tests among the vaccinated and the unvaccinated women are described in Table 3. The HC 2 test positivity (for any of the 13 high risk types) in the 432 women who received two doses at 15–18 years was comparable to the HC 2 positivity in 409 women who received three doses at the same age (3.9% vs. 4.2%). The HC 2 was positive in 5.7% of the 3511 unvaccinated women. The proportion of HC 2 positive women also positive for HPV 16 and/or HPV 18 on HC 2 genotyping was 0.9% in the 2-dose, age 15–18 cohort, 0.2% in the 3-dose, age 15–18 cohort, and 1.3% in the unvaccinated women.

At the time of this analysis, data on disease confirmation in the vaccinated cohorts was available for only the women who received the three doses or two doses at age 15–18 years. Of the 34 women in the vaccinated cohorts positive on HC 2, 24 (70.6%) had colposcopy and/or biopsy by the time of this analysis. No CIN 2 or worse lesions were detected in both cohorts of the women who received the two doses or three doses of the vaccine at 15–18 years. In the unvaccinated cohort, 132 (66.0%) of the 200 HC 2 positive women had disease verification

Table 3
Hybrid capture 2 test positivity by HPV vaccine dose received and age group.

Vaccine dose received	Age at first vaccine dose (years)	Women screened	HC 2 positivity																	
			Overall		HPV 16 alone		HPV 18 alone		HPV 45 alone		HPV 16 and 18		HPV 16 and 45		HPV 18 and 45		HPV 16, 18 and 45		Other high-risk HPV types excluding 16, 18 and 45	
		n	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
3-dose (Days 1, 60 and ≥ 180)	10–14	3	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	15–18	409	17	(4.2)	0	(0.0)	0	(0.0)	1	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	16	(3.9)
2-dose (Days 1 and ≥ 180)	10–14	4	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	15–18	432	17	(3.9)	0	(0.0)	1	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.2)	2	(0.5)	13	(3.0)
Unvaccinated group		3511	200	(5.7)	21	(0.6)	13	(0.4)	4	(0.1)	3	(0.1)	0	(0.0)	0	(0.0)	2	(0.1)	157	(4.5)

HPV: human papilloma virus.

by colposcopy/biopsy. In these HC 2 positive unvaccinated women, two CIN 2 and two CIN 3 lesions were detected. Both CIN 2 and one of the CIN 3 lesions were positive for HPV 16 and/or 18 infections using the HC 2 genotyping test (Table 4).

4. Discussion

The WHO guidance recommending two doses for young adolescents below 15 years of age was based on the outcomes of the immunological bridging studies that traditionally selected 9–14 years age group to be compared with the older women with a broad age range of 15–26 years; the efficacy of the three doses of the vaccine being proven in the later age group [3,10–13]. Some of these studies also demonstrated the immunogenic non-inferiority of two doses in the 9–14 year old girls compared to the standard of care three doses in the same age. The only other study to include the age-stratified immunogenicity data for the 15–18 year old girls was the one by Romanowski et al., which demonstrated the non-inferiority of two dose schedule of the bivalent vaccine compared to a three dose schedule in 15–19 year old girls [10].

We have reported in our earlier publication that all the girls receiving two or three doses of the vaccine seroconverted at 1 month after vaccination irrespective of age. The L1 antibody titres in the 15–18 years old girls receiving two doses were non-inferior for all four vaccine targeted HPV types when compared to the 15–18 years old girls receiving three doses of the vaccine, at 7 months (1 month after the last dose), 36 months and 48 months after the first dose [7]. The neutralizing antibody titres against HPV 16 and HPV 6 at 18 months in the 15–18 years old 2-dose group were non-inferior to that in the girls of same age receiving three doses. The neutralizing antibody titres against HPV 18 were inferior in the 2-dose recipients and the HPV 11 titres were not estimated. However, the immune correlate of protection of the antibodies against HPV being still unknown, the true significance of lower anti-HPV 18 neutralizing antibody titre should be assessed in the context of the protection against infection and/or disease.

In the present manuscript we report the significant protection against incident as well as persistent infections from the vaccine-targeted HPV types being offered by two doses of the quadrivalent vaccine in the girls receiving the vaccine at 15–18 years of age. This is expected out of the strong, durable and consistent antibody response already observed after two doses at this age. Based on the analysis of nearly 1500 women providing serial cervical samples each in 2- and 3-dose groups, we have established that the protection against incident infections (from vaccine targeted types as well as the cross protective types) in the 2-dose recipients at 15–18 years of age remains same as the standard of care groups (2-dose recipients at 10–14 years and 3-dose recipients at 15–18 years). Persistent infection being a more appropriate surrogate for cervical neoplasia, the protection against persistent infection from the vaccine targeted HPVs reported in our study with two doses irrespective of age at vaccination makes a strong argument to extend the two dose recommendation to the 15–18 years old girls.

Our efficacy data corroborate the observations of the study nested within the phase III RCT in Costa Rica to evaluate the efficacy of two doses of bivalent vaccine. The proportion of women with persistent HPV 16 and/or HPV 18 infections (defined as the detection of same HPV type in two consecutive samples obtained at least 10 months apart) was 0.85% (95% CI 0.56–1.2%) and 0.71% (95% CI 0.18–1.9%) in the 3-dose and 2-dose recipients respectively [14]. The high vaccine efficacy of two doses was observed in the Costa Rican study even if age at vaccination was 18–25 years and the two doses were administered at an interval of one month. Vaccination with two doses at a lower age is expected to offer at least similar protection, if not better.

We have also demonstrated the low detection of HPV 16 and HPV 18 infection using another validated HPV detection test in recipients of the HPV vaccine irrespective of the number of doses and the age at vaccination. Though the number of high grade CIN detected in the vaccinated or the unvaccinated cohorts was too small to comment on vaccine efficacy, the trends show protection against HPV 16/HPV 18 induced lesions in the two- or three-dose recipients at 15–18 years of age.

The IARC/NCI Expert Group unanimously agreed that immunobridging based on non-inferiority of the immune response using a standardized immunological test was a valid approach to recommend alternative schedules/doses for groups older (or younger) than 9–15 years [2]. Our study provides both the immune-bridging data as well as the efficacy data against persistent infections to favour the 2-dose recommendation for the 15–18 years old girls. This will allow countries to vaccinate multiple age cohorts up to 18 years with two doses at introduction, thus saving resources and making the program more cost-effective. In fact, Colombia has extended the target age for HPV vaccination for girls up to 17 years with two doses [15]. Many other countries can follow the same example with a formal recommendation from the WHO, thus achieving a higher impact of HPV vaccination and accelerating progress towards cervical cancer elimination.

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Table 4
Final diagnosis among HC 2 positive women by number of vaccine doses received.

Vaccine dose received	Women positive on HC 2 test	Final diagnosis available among women positive on HC 2 test[a]		CIN diagnosed among all women positive on HC2 test			CIN diagnosed among women positive on HC 2 for HPV types 16 and/or 18		
				CIN 1	CIN 2	CIN 3	CIN 1	CIN 2	CIN 3
	n	n	(%)	n	n	n	n	n	n
Women vaccinated with 2-dose or 3-dose	34	24	(70.6)	1	0	0	0	0	0
3-dose (Days 1, 60 and ≥180)	17	15	(88.2)	1	0	0	0	0	0
2-dose (Days 1 and ≥180)	17	9	(52.9)	0	0	0	0	0	0
Unvaccinated group	200	132	(66.0)	10	2	2	3	2	1

HPV: human papilloma virus; HC 2: hybrid capture 2; CIN: cervical intraepithelial neoplasia.
[a] Currently all women with confirmed diagnosis are for the age group 15-18.

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Conflicts of interest

Neerja Bhatla has received research funding through her institute from GlaxoSmithKline and Merck. Smita Joshi has received funds from GlaxoSmithKline through the Jehangir Clinical Development Center to do an HPV vaccine study. Partha Basu received research funding from GlaxoSmithKline through Chittaranjan National Cancer Institute, India during his previous position at the institute. The other authors declare no competing interests. Michael Pawlita is an inventor on a patent application by DKFZ on the high-throughput pseudovirion-based neutralisation assay. Peter Sehr is co-inventor on a patent application by DKFZ on the high-throughput pseudovirion-based neutralisation assay.

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