Vaccine 41 (2023) 236–245

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Evaluation of immune response to single dose of quadrivalent HPV vaccine at 10-year post-vaccination

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ARTICLE INFO

Article history:
Received 10 June 2022
Received in revised form 2 August 2022
Accepted 19 November 2022
Available online 26 November 2022

Keywords:
Human papillomavirus
quadrivalent HPV vaccine
Single dose
Vaccine immune responses
Natural immunity

ABSTRACT

Background: The recent World Health Organization recommendation supporting single-dose of HPV vaccine will significantly reduce programmatic cost, mitigate the supply shortage, and simplify logistics, thus allowing more low- and middle-income countries to introduce the vaccine. From a programmatic perspective the durability of protection offered by a single-dose will be a key consideration. The primary objectives of the present study were to determine whether recipients of a single-dose of quadrivalent HPV vaccine had sustained immune response against targeted HPV types (HPV 6,11,16,18) at 10 years post-vaccination and whether this response was superior to the natural antibody titres observed in unvaccinated women.

Methods: Participants received at age 10–18 years either one, two or three doses of the quadrivalent HPV vaccine. Serology samples were obtained at different timepoints up to 10 years after vaccination from a convenience sample of vaccinated participants and from age-matched unvaccinated women at one timepoint. The evolution of the binding and neutralizing antibody response was presented by dose received. 10-year durability of immune responses induced by a single-dose was compared to that after three doses of the vaccine and in unvaccinated married women.

Results: The dynamics of antibody response among the single-dose recipients observed over 120 months show stabilized levels 18 months after vaccination for all four HPV types. Although the HPV type-specific (binding or neutralizing) antibody titres after a single-dose were significantly inferior to those after three

Abbreviations: HPV, Human papillomavirus.

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https://doi.org/10.1016/j.vaccine.2022.11.044
0264-410X/

doses of the vaccine (lower bounds of GMT ratios < 0.5), they were all significantly higher than those observed in unvaccinated women following natural infections (GMT ratios: 2.05 to 4.04-fold higher). The results correlate well with the high vaccine efficacy of single-dose against persistent HPV 16/18 infections reported by us earlier at 10-years post-vaccination.

Conclusion: Our study demonstrates the high and durable immune response in single-dose recipients of HPV vaccine at 10-years post vaccination.

1. Introduction

Vaccines against human papillomavirus (HPV) has been introduced in the national immunization programme in 55 countries till June 2020 [1]. Only 41 % of the low- and middle-income countries (LMICs) have been able to introduce the vaccines even though they bear approximately 85 % of the global burden of new cervical cancer cases. The huge inequity in access to the potentially lifesaving vaccines is underlined by the fact that only 15 % of girls globally received complete doses of the vaccines till 2019. HPV vaccines are one of the most expensive vaccines and two doses of the vaccines may be unaffordable to many of the LMICs even at the current Gavi price of $4.50 US per dose [2]. While following introduction of the vaccines, LMICs achieved a better first dose coverage compared to high income countries (mean coverage 80 % versus 72 %, respectively), the dropout rate for the second dose was significantly higher in the LMICs, highlighting the logistic challenges of administering a second dose in limited resourced settings [1]. In recent years a supply crisis of HPV vaccines has forced the World Health Organization (WHO) to recommend withholding vaccination of multi-age cohort [3]. The COVID-19 pandemic has significantly disrupted HPV vaccination even in high income countries; administration of the second dose being worse affected [4]. In this context, the recent WHO recommendation for countries to use either a single or two doses of the vaccines for recipients up to 20 years will have a significant public health impact [5]. The WHO recommendation was based on evidence generated from several observational and ecological studies and one randomized controlled trial [6–9]. The evidence was also considered to be robust and compelling enough by the vaccine advisory committee of the United Kingdom to recommend switching to a single dose in their national immunization programme [10].

From a programmatic perspective, the durability of protection offered by a single dose will be a key consideration while considering adoption of this new dose recommendation. Durability is dependent on the length of time over which the type-specific antibody is sustained at a level adequate to offer protection against persistent HPV infection. Earlier studies have demonstrated that, despite being inferior to the levels observed after two or three doses, the level of antibody after a single dose of Cervarix™ remained stable throughout a follow-up of 11 years [7]. The antibody titre was significantly higher compared to pre-vaccination levels in participants with natural infection and the vaccine efficacy against incident persistent HPV 16/18 remained high at least through the duration of follow up. Long term immunogenicity data is not yet available for the quadrivalent or the nonavalent vaccines.

The primary objectives of the present study were to determine whether recipients of a single dose of quadrivalent HPV vaccine had sustained immune response against targeted HPV types (HPV 6,11,16,18) at 10 years post-vaccination and whether this response was superior to the natural immunity observed in unvaccinated women.

The secondary objective was estimation of the immune response against vaccine-targeted HPV types at 10 years post-vaccination in the recipients of three doses and two doses of the quadrivalent HPV vaccine.

2. Methods

2.1. Recruitment of study subjects

A cluster-randomized trial was initiated in September 2009 by the International Agency for Research on Cancer (IARC), France in nine centers in India to compare the effectiveness of two doses (administered on days 1 and 180) and three doses (administered on days 1, 60 and 180) of quadrivalent HPV vaccine (Gardasil™; Merck Sharp & Dohme, NJ, USA). The target was to recruit 20,000 unmarried girls aged 10 to 18 years equally divided between the two-dose and three-dose arms. Due to some events unrelated to our study, the Indian Government issued an administrative order on 9th April 2010 to suspend HPV vaccination in all research studies in the country, which affected our study as well. Till then the study had recruited 17,729 girls, randomly allocated to receive either two or three doses of the vaccine. Abrupt stoppage of vaccination resulted in the girls receiving different vaccine schedules as follows: 4,348 girls received three doses on days 1, 60 and $\geq$ 180, 4,979 received two doses on days 1 and $\geq$ 180, 3452 received two doses on days 1 and 60 by default, and 4,950 received one dose by default. Due to lack of clinical relevance, the two-dose default group (receiving vaccine at days 1 and 60) will not be discussed any further in this manuscript. The two-dose and three-dose groups described here includes only those vaccinated at days 1 and 180 and days 1, 60, and 180, respectively. Unvaccinated married women (N = 1,484) matched by age and place of residence to the vaccinated 18- to 21-year-old married participants were recruited post-hoc as a control group during 2013–15. All the study participants are being followed up yearly. The details of the follow up protocol and vaccine efficacy by dose groups have been published earlier [6].

2.2. Collection of blood samples to assess immunogenicity

A serology cohort, comprised of a convenience sample of participants representing all included ages, provided blood samples at baseline and subsequent timepoints for immunogenicity analysis. The participants receiving two or three doses of the vaccine provided samples at 7 months according to protocol. Blood samples could be collected from the single dose recipients only at 12 months after vaccination due to disruption of dosage schedule. Subsequent blood samples were collected at months 24, 36, 48 and 60 from the serology cohort according to the original protocol. In addition, samples were collected from nearly all study participants at month 18.

Blood sample collection for the 10-year immunogenicity study was initiated in February 2020 and completed by July 2020. Randomly selected 340 single dose recipients belonging to the serology cohort and providing at least at baseline and 12 months post-vaccination samples were invited to participate, and 324 pro-

vided a blood sample. Blood samples were also requested from 200 randomly selected serology cohort participants in each of the two-dose and three-dose groups, with 190 and 167, respectively, providing samples. Blood samples were also collected from 352 randomly selected unvaccinated women. All the participants providing additional samples were reconsented.

A trained phlebotomist collected 10 mL of blood from the consenting participants following standard aseptic precautions. The blood samples were treated with EDTA, plasma was separated, and multiple aliquots were made and stored in in cryovials before being transported at −20 °C to the centralized laboratory facility at Rajiv Gandhi Center for Biotechnology, Trivandrum, India for storage in a deep freezer at −80 °C.

To evaluate stability of the immune response, stored plasma aliquots collected at earlier time points from women providing a 10-year sample were retrieved. Stored samples and those collected for the 10-year immunogenicity study were shipped on dry ice to testing laboratories. Details of the samples tested and included in this analysis are given in Table 1.

2.3. Analysis of samples using multiplex VLP-based IgG ELISA (M9ELISA)

Detection and quantitation of type-specific antibodies to HPV 6, 11, 16 and 18 was performed using the Multiplex VLP-based IgG ELISA (M9ELISA) at the Centers for Disease Control and Prevention (CDC), Atlanta, GA USA, as previously described [11]. For quality control 5 % of the samples were repeat tested. The laboratory personnel were blinded to the vaccination status and other personal information of the participants providing samples. The parallel line model (PLL) analysis was performed as described in the World Health Organization (WHO) HPV Labnet Manual [12]. The PLL value is the amount of antibody present in the test serum relative to the standard/reference serum used [13]. Three dilutions of raw relative light units (RLU) for each sample were used in the analysis. To pass the PLL conditions all test samples had to have a correlation $\geq$ 0.9, slope $\leq$ -0.4, slope ratio $\geq$ 0.5 and at least 2 data points within linear range compared to the reference sample. The binding antibody titres were reported in International Units/mL (IU/mLmL) for HPV 16 and 18, and in arbitrary units/mL (AU/mLmL) for HPV 6 and 11.

The reproducibility of PLL values generated for the controls was maintained on a Levy Jennings plot throughout the course of the study. The HPV type-lower limit of quantitation (LLOQ) representing the cut-off for detectable antibodies was established following validation [11,14]. The values of the LLOQ were 1.0 IU/mLmL for HPV 16, 0.3 IU/mL for HPV 18, 0.2 AU/mL for HPV 6 and 0.4 AU/mL for HPV 11.

2.4. Analysis of samples using pseudovirion-based neutralization assay (PBNA)

Antibodies specific to neutralizing epitopes in HPV-L1 protein of HPV 6, 11, 16 and 18 were measured with a highly sensitive, automated, high-throughput PBNA at the joint Chemical Biology Core Facility of the European Molecular Biology Laboratory (EMBL) and the German Cancer Research Center (DKFZ; Heidelberg, Germany) [15]. It is considered the "gold-standard" of functional humoral immune responses to HPV. The laboratory personnel were blinded to the vaccination status and other details of the participants providing samples. The neutralization antibody titres were reported in International Units/ml (IU/mL) for HPV 16 and 18, and in arbitrary units/ml (AU/mL) for HPV 6 and 11. The cut-offs for the lowest detectable titres were 40 IU/mL for HPV 16 and HPV 18, 155 AU/mL for HPV 6 and 156 AU/mL for HPV 11.

2.5. Sample size estimation based on the primary objective

The sample size estimates for the primary outcome of total immune responses were calculated for the two cohorts relevant for the primary objective, single dose and unvaccinated. With the assumptions that the antibody levels in the single dose group are elevated by a factor of 1.5 as compared to those in the unvaccinated group, log standard deviation of 1.5, a 90 % power and 5 % two-sided level of significance, the required sample size in each group was 289 participants. For subgroup analyses taking into consideration the α adjustments for two age strata (20–24 and 25–28 years) approximately 340 participants were required in each group (single-dose and unvaccinated). The sample size in the two-dose and three-dose groups were empirically decided.

2.6. Outcomes

The two primary outcomes considered were: the HPV genotype-specific binding antibody titres using geometric mean PLL; and HPV type-specific neutralization antibodies using geometric mean titres (GMTs). In this analysis we present titres only for the four vaccine-targeted HPV types 16, 18, 6 and 11. The binding antibody outcome was assessed at the following time points: day 1, month 7 after first vaccination dose (for three-dose group only), month 12 (for single-dose group only), months 18 and 36 (for single- and three-dose groups), and month 120 (for single-, two- and three-dose groups). The neutralization antibody outcome

Table 1
Number of samples tested and included in the analysis of the binding and neutralization immune responses.

Time of	Tested on M9ELISA					Tested on PBNA				
Sample	Unvaccinated	Vaccinated cohort				Unvaccinated	Vaccinated cohort			
collection	cohort	Any	Single-dose	Two- dose (days 1 and 180)	Three- dose (days 1, 60 and 180)	cohort	Any	Single-dose	Two- dose (days 1 and 180)	Three- dose (days 1, 60 and 180)
Not defined	352					351				
Day 1		150					170			
Month 7					154					120
Month 12			150							
Month 18			148		154			58	59	60
Month 24								60		
Month 36			150		136				59	60
Month 48								60	39	34
Month 60								34	179	176
Month 120			324	190	167			324	190	167

ELISA: enzyme-linked immunosorbent assay; PBNA: pseudovirion-based neutralisation assay

was assessed at the following time points: day 1, month 7 after first vaccination dose (for three-dose group only), month 18 (for single-, two- and three-dose groups), month 24 (for single-dose group only), month 36 (for two- and three-dose groups), and months 48, 60 and 120 (for single-, two- and three-dose groups). HPV 11 neutralization titres were tested at months 60 and 120 time points only.

2.7. Statistical analysis

The HPV type-specific proportion of participants with detectable titres was presented for the different study groups. The HPV type-specific mean antibodies were calculated using data of participants with detectable antibodies. The antibody response levels for the single- and two-dose groups were compared with those induced in the three-dose group. The antibody titres of the different vaccine recipients were additionally compared to those from natural immunity in the unvaccinated cohort. These comparisons were done using log-transformed mean titres in linear regression models to obtain GMT ratios and their corresponding 95 % confidence intervals (CIs). Non-inferiority of the single- and two-dose groups compared to the three-dose group was concluded if the lower bound of the 95 % CI for its GMT ratio was greater than 0.5 In keeping with other HPV immunogenicity studies [16,17]. For comparisons with the unvaccinated cohort, 5 % was taken as the level of significance. All statistical analyses were done using Stata (version 17.0).

The study has been approved by the ethics committee of IARC and all participating institutions. The trial is registered with ISRCTN (ISRCTN98283094), and ClinicalTrials.gov (NCT00923702).

3. Results

The mean age at the time of 10-year sample collection among the 324 single dose recipients was 23.7 (±2.5) years, 190 two-dose recipients was 23.8 (±2.6) years, and 167 three-dose recipients was 23.8 (±2.5) years. The mean age of the 352 unvaccinated participants was 24.6 (±2.2) years when a single time blood collection was done.

The details of the immune response assessed with M9ELISA against vaccine targeted types at different time points are shown in Table 2. Geometric mean titre (GMT) of antibody against HPV 16 among the single dose recipients at 12-month post-vaccination was 9.72 IU/mL (95 %CI 8.30 to 11.37). At 120 months the GMT was almost unchanged (9.90 IU/mL; 95 %CI 8.76 to 11.19) with 96.0 % of the single dose recipients having detectable antibody against HPV 16. The HPV 16 antibody titre in this group was 2.05 times (95 %CI 1.34 to 3.16) higher compared to that observed in the unvaccinated participants, but significantly inferior compared to both two-dose and three-dose groups. The ratio of GMT in the single dose compared to three dose recipients was 0.28 (95 %CI 0.24 to 0.33).

The dynamics of antibody response observed against HPV 18, HPV 6 and HPV 11 in the single-dose recipients over the 120 months closely mimicked the pattern observed for antibody against HPV 16 (Fig. 1). The GMT against HPV 18 in the single dose recipients was 3.27 IU/mL (95 %CI 2.77 to 3.86) and 2.58 IU/mL (95 %CI 2.26 to 2.95) at months 12 and 120 respectively (Table 2). HPV 18 antibody was detectable in 96.9 % of the single dose recipients at 120 months, and the GMT was 3.47 times (95 %CI 2.68 to 4.51) higher compared to that detected in the unvaccinated women. The HPV 18 antibody titre in single dose group was significantly inferior compared to that observed in the three-dose group (GMT ratio 0.32; 95 %CI 0.26 to 0.39).

At 120 months post-vaccination the GMT of antibody against HPV 6 was 4.04 times (95 %CI 3.08 to 5.31) and against HPV 11 was 2.79 times (1.54 to 5.05) higher in the single dose group compared to the unvaccinated women. Antibody against HPV 6 and HPV 11 was detected in 96.9 % and 93.5 % of the single dose recipients respectively at 120 months.

The evolution of neutralization antibody response (assessed by PBNA) against HPV 16, HPV 18, HPV 6 and HPV 11 at 120 months post-vaccination was very similar to the antibody response observed by M9ELISA against the corresponding HPV type (Fig. 2). The neutralizing GMT against HPV 16 following a single dose administration at 18 months was 558 (95 %CI 416 to 750) (Table 3). The same at 120 months was 819 (95 %CI 701 to 957) with 97.8 % of the participants having detectable neutralizing antibody. The mean neutralization GMT against HPV 16 at 120 months in the single dose recipients was 2.14 times (95 %CI 1.19 to 3.84) higher than that of unvaccinated women. The mean neutralization GMT ratio between the single-dose group and the three-dose group at 120 months post-vaccination was 0.25 (0.18 to 0.35).

The mean neutralizing antibody titre against HPV 18 in the single dose recipients was 156 (95 %CI 113 to 216) at 18 months with 67.2 % of the samples having detectable neutralization GMT. The titre remained stable at 120 months (172; 95 %CI 146 to 204) with 49.1 % of the samples having detectable antibody. The neutralizing antibody titre in single dose recipients was significantly lower compared to three dose recipients (ratio 0.67; 95 % CI 0.49 to 0.92), though 2.05 times higher compared to that observed in unvaccinated women.

Compared to 48 months an appreciable (though statistically non-significant) increase in the neutralizing GMT was observed in the single dose group at 120 months against both HPV 16 [388 (95 %CI 284 to 530) vs 819 (95 %CI 701 to 957)] and HPV 18 [105 (95 %CI 73 to 151) vs 172 (95 %CI 146 to 204)]. A similar rise was also noted in the mean total antibody titre on M9ELISA against HPV 16 and HPV 18 between month 36 and month 120.

4. Discussion

Our study demonstrates that both total and neutralizing antibody titres against the vaccine targeted HPV types plateau between 12 and 18 months after administration of a single dose of quadrivalent vaccine and remain almost constant since then till at least 120 months. The antibody against any of the four quadrivalent vaccine types (HPV 6/11/16/18) remains detectable at 120 months in high proportion of the single dose recipients, and at a level several fold higher than that observed with natural immunity. The long-term immunogenicity observed in the present study correlates very well with the high vaccine efficacy of single dose against persistent HPV 16 and 18 infections (95.4 %; 95 %CI 85.0 to 99.9) or against persistent infection from any of the four quadrivalent vaccine types (93.4 %; 81.1 to 99.1) reported by us earlier at 10 years post vaccination [6].

Our results are very similar to the 11-year immunogenicity outcomes reported by the Costa Rica HPV Vaccine Trial (CVT), in which 18–25 year old women received various doses of bivalent vaccine (Cervarix™, GSK Biologicals) [7]. The protocol called for a three-dose series, but some women received only one or two doses. The HPV16 or HPV 18 antibody levels remained stable at 11-years post-vaccination among women who received a single dose with seropositivity being 96.7 % (95 %CI: 93.3 % to 98.7 %) for HPV16 and 92.9 % (95 %CI: 88.5 % to 96.0 %) for HPV18 on virus like particle (VLP)-based enzyme-linked immunosorbent assay (ELISA). The similarity of long-term immunogenicity and vaccine efficacy outcomes between CVT and our study reinforces the fact that a sin-

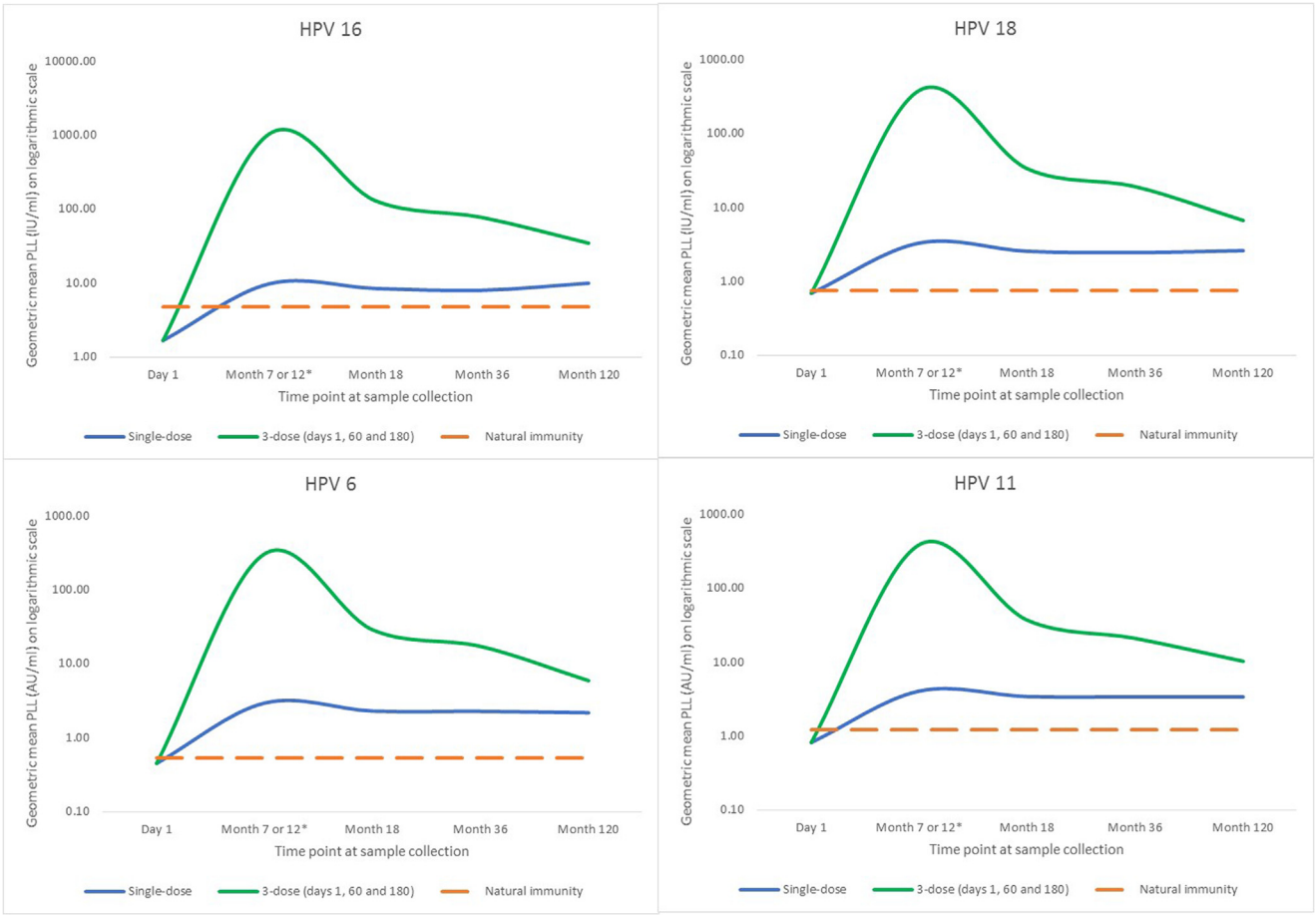

Fig. 1. Evolution of immune responses for vaccine-targeted HPV types 16, 18, 6 and 11 obtained using M9ELISA assay over time in the recipients of a single-dose or three doses of the HPV vaccine compared to the antibody levels detected in unvaccinated participants (natural immunity) *[*blood was collected from three dose recipients at 7 months and from single dose recipients at 12 months after dose 1].*

gle dose of the vaccines can provide durable protection that is unlikely to wane over several years.

We hypothesized that the antibody levels in the single dose group would be elevated by a factor of 1.5 as compared to those in the unvaccinated group as an assumed guess, since no previous studies had reported comparisons of immune responses between the single dose recipients and naturally infected women for the quadrivalent HPV vaccine, and the Costa Rican study had consistently reported higher antibody titres among the recipients of bivalent HPV vaccine (Cervarix™, GSK Biologicals) compared to that elicited by natural HPV infections several years after vaccination [7]. We observed 2-fold elevated immune responses for the single-dose compared to natural infection in this study, which implied our study had enough sample size and power even at a factor of 2.

Switching from two doses of the HPV vaccines to a one dose based on the latest WHO recommendation will significantly reduce the programmatic cost, improve affordability and supply, and simplify the logistics, thus allowing more LMICs to introduce the vaccines. The resources and vaccine doses saved may be used to vaccinate multi-age cohorts and even boys resulting in faster elimination of cervical cancer. Modelling studies have shown that single dose vaccination of young adolescent girls would always be cost saving compared to no vaccination in the LMIC setting, and significantly greater health benefit would be expected from single dose, if protection did not wane at least till 15 years [18] . Based on findings in our study and the CVT study it is likely that the antibody titres and protection from a single dose will continue to persist through at least 15 years. Assuming a 70 % coverage and 80 % efficacy of the vaccines against HPV 16/18 compared to no vaccination, the modelling study demonstrated that single dose vaccination over no vaccination would avert between 400,000 and 550,000 DALYs (disability adjusted life years) over the lifetimes of the current population of Ugandan women (Ugandan population data was used for modeling) under age 50. Our study suggests that the vaccine efficacy even beyond 10 years is likely to exceed 90 %; thus, the quantum of benefit will be substantially higher than that projected by the model.

Mechanistic studies have established the biological plausibility of a single dose of HPV vaccines being almost as effective as higher number of doses. The HPV vaccine antigens are L1 proteins self-assembled to virus like particles (VLP) that are structurally and immunochemically similar to authentic virions. While sub-unit vaccines are traditionally administered in a series of prime-boost doses, the highly ordered and closely packed 3-dimensional structure of HPV L1 VLPs induce high and durable titres of binding antibodies with a single dose. The densely ordered repetitively displayed B cell epitopes on the surface of the VLPs bind to the naïve B cells that in turn leads to strong induction of the long-lived plasma cells (LLPCs) residing primarily in the bone marrow [19][20]. The LLPCs are responsible for producing the type-specific antibodies over many years.

Even if the antibody levels induced by a single dose is significantly inferior to that seen following administration of two or

Table 2
Distribution and comparison of immune responses for vaccine-targeted HPV types 16, 18, 6 and 11 obtained using M9ELISA assay by dose received and time of sample collection.

	No. of samples	No. with detectable antibody titres* (%)		GMT (95 % CI)**			GMT ratio (95 % CI) Alternate/3-dose$			GMT ratio (95 % CI) Dose received/Unvaccinated		
HPV 16												
Unvaccinated cohort	352	27	(7.7)	4.82	(3.23 -	7.19)				1.00		
Day 1												
Vaccinated cohorts	150	7	(4.7)	1.67	(1.46 -	1.92)				0.35	(0.16 -	0.76)
Month 7												
3-dose (days 1, 60 and 180)	154	154	(100.0)	1045.37	(917.45 -	1191.13)				216.87	(152.80 -	307.80)
Month 12												
Single-dose	150	148	(98.7)	9.72	(8.30 -	11.37)				2.02	(1.35 -	3.02)
Month 18												
Single-dose	148	146	(98.6)	8.40	(7.07 -	9.98)	0.06	(0.05 -	0.08)	1.74	(1.13 -	2.69)
3-dose (days 1, 60 and 180)	154	154	(100.0)	129.36	(113.12 -	147.94)	1.00			26.84	(18.76 -	38.39)
Month 36												
Single-dose	150	147	(98.0)	7.94	(6.66 -	9.46)	0.10	(0.07 -	0.16)	1.65	(1.06 -	2.56)
3-dose (days 1, 60 and 180)	136	136	(100.0)	77.27	(65.63 -	90.98)	1.00			16.03	(10.70 -	24.01)
Month 120												
Single-dose	324	311	(96.0)	9.90	(8.76 -	11.19)	0.28	(0.24 -	0.33)	2.05	(1.34 -	3.16)
2-dose (days 1 and 180)	190	190	(100.0)	34.74	(30.40 -	39.70)	0.98	(0.83 -	1.17)	7.21	(4.92 -	10.56)
3-dose (days 1, 60 and 180)	167	167	(100.0)	35.40	(30.44 -	41.16)	1.00			7.34	(4.89 -	11.01)
HPV 18												
Unvaccinated cohort	352	95	(27.0)	0.74	(0.62 -	0.88)				1.00		
Day 1												
Vaccinated cohorts	150	49	(32.7)	0.69	(0.58 -	0.83)				0.93	(0.71 -	1.22)
Month 7												
3-dose (days 1, 60 and 180)	154	154	(100.0)	379.86	(324.21 -	445.07)				511.10	(400.94 -	651.52)
Month 12												
Single-dose	150	150	(100.0)	3.27	(2.77 -	3.86)				4.40	(3.43 -	5.64)
Month 18												
Single-dose	148	144	(97.3)	2.53	(2.10 -	3.05)	0.08	(0.05 -	0.12)	3.40	(2.60 -	4.46)
3-dose (days 1, 60 and 180)	154	154	(100.0)	33.22	(27.85 -	39.62)	1.00			44.69	(34.40 -	58.07)
Month 36												
Single-dose	150	147	(98.0)	2.44	(2.05 -	2.90)	0.13	(0.08 -	0.21)	3.28	(2.53 -	4.24)
3-dose (days 1, 60 and 180)	136	136	(100.0)	19.03	(15.39 -	23.54)	1.00			25.61	(19.13 -	34.29)
Month 120												
Single-dose	324	314	(96.9)	2.58	(2.26 -	2.95)	0.32	(0.26 -	0.39)	3.47	(2.68 -	4.51)
2-dose (days 1 and 180)	190	186	(97.9)	6.64	(5.58 -	7.90)	0.82	(0.65 -	1.02)	8.93	(6.80 -	11.74)
3-dose (days 1, 60 and 180)	167	167	(100.0)	8.13	(6.78 -	9.75)	1.00			10.94	(8.32 -	14.38)
HPV 6												
Unvaccinated cohort	352	73	(20.7)	0.54	(0.43 -	0.67)				1.00		
Day 1												
Vaccinated cohorts	150	34	(22.7)	0.45	(0.35 -	0.56)				0.83	(0.58 -	1.19)
Month 7												
3-dose (days 1, 60 and 180)	154	154	(100.0)	305.04	(264.10 -	352.33)				567.52	(438.52 -	734.47)
Month 12												
Single-dose	150	150	(100.0)	2.94	(2.50 -	3.47)				5.48	(4.14 -	7.25)
Month 18												
Single-dose	148	148	(100.0)	2.29	(1.93 -	2.72)	0.08	(0.06 -	0.11)	4.26	(3.20 -	5.68)
3-dose (days 1, 60 and 180)	154	154	(100.0)	28.62	(24.41 -	33.55)	1.00			53.24	(40.42 -	70.13)
Month 36												
Single-dose	150	149	(99.3)	2.27	(1.90 -	2.72)	0.13	(0.08 -	0.21)	4.22	(3.13 -	5.70)
3-dose (days 1, 60 and 180)	136	136	(100.0)	17.07	(14.03 -	20.76)	1.00			31.75	(23.25 -	43.36)
Month 120												
Single-dose	324	314	(96.9)	2.17	(1.92 -	2.45)	0.27	(0.22 -	0.34)	4.04	(3.08 -	5.31)
2-dose (days 1 and 180)	190	190	(100.0)	5.87	(5.01 -	6.87)	0.74	(0.58 -	0.94)	10.92	(8.18 -	14.57)
3-dose (days 1, 60 and 180)	167	167	(100.0)	7.92	(6.73 -	9.32)	1.00			14.74	(11.08 -	19.61)
HPV 11												
Unvaccinated cohort	352	13	(3.7)	1.21	(0.78 -	1.87)				1.00		
Day 1												
Vaccinated cohorts	150	4	(2.7)	0.82	(0.33 -	2.05)				0.68	(0.29 -	1.58)
Month 7												
3-dose (days 1, 60 and 180)	154	154	(100.0)	383.15	(330.91 -	443.65)				317.18	(188.98 -	532.33)
Month 12												
Single-dose	150	147	(98.0)	4.04	(3.34 -	4.89)				3.35	(1.74 -	6.43)
Month 18												
Single-dose	148	140	(94.6)	3.41	(2.84 -	4.09)	0.09	(0.07 -	0.12)	2.82	(1.53 -	5.21)
3-dose (days 1, 60 and 180)	154	154	(100.0)	36.70	(31.35 -	42.97)	1.00			30.38	(17.44 -	52.92)
Month 36												
Single-dose	150	142	(94.7)	3.38	(2.80 -	4.07)	0.16	(0.11 -	0.25)	2.79	(1.48 -	5.27)
3-dose (days 1, 60 and 180)	136	136	(100.0)	20.81	(17.09 -	25.34)	1.00			17.23	(8.99 -	33.00)
Month 120												
Single-dose	324	303	(93.5)	3.37	(2.99 -	3.81)	0.33	(0.28 -	0.40)	2.79	(1.54 -	5.05)
2-dose (days 1 and 180)	190	188	(98.9)	10.21	(8.85 -	11.79)	1.01	(0.79 -	1.30)	8.46	(4.85 -	14.73)

(*continued on next page*)

Table 2 (*continued*)

	No. of samples	No. with detectable antibody titres* (%)	GMT (95 % CI)**	GMT ratio (95 % CI) Alternate/3-dose$	GMT ratio (95 % CI) Dose received/Unvaccinated
3-dose (days 1, 60 and 180)	167	167 (100.0)	10.11 (8.61 - 11.88)	1.00	8.37 (4.65 - 15.07)

GMT: geometric means titres; CI: confidence interval; HPV: human papilloma virus; * The values of the lower limit of quantitation (LLOQ) were 1.0 for HPV 16, 0.3 for HPV 18, 0.2 for HPV 6 and 0.4 for HPV 11. ** The HPV type-specific mean antibodies were calculated using data of participants with detectable antibodies. International Units/ml (IU/mL) for HPV types 16 and 18 and arbitrary units/ml (AU/mL) for HPV types 6 and 11; $ Other dose schedules were non-inferior to the 3-dose schedule if the lower bound of the 95 % CI for the GMT ratio was above 0.5 (2-fold difference).

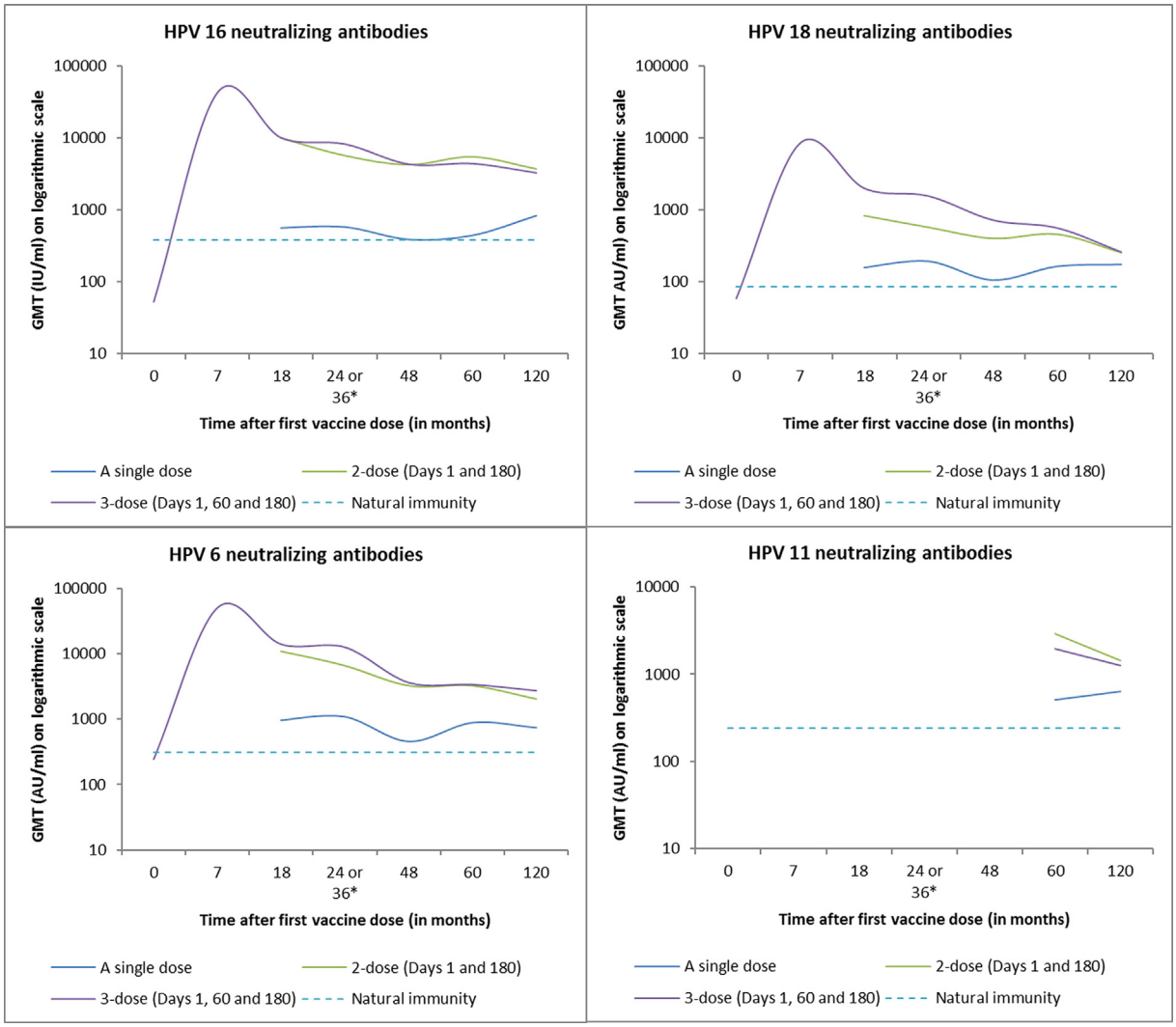

Fig. 2. Evolution of neutralization geometric mean titres for HPV 16, 18, and 11 obtained using PBNA assay over time in the recipients of a single-dose, two or three doses of the HPV vaccine compared to the antibody levels detected in unvaccinated participants (natural immunity) *[*blood was collected from single dose recipients at 24 months and from two/three dose recipients at 36 months after dose 1. HPV 11 neutralization titres were tested at months 60 and 120 time points only].*

three doses (as shown in our study), the protection is not compromised due to the unique natural history of the papillomavirus. The small amount of antibody exuded at the site of microtraumas, through which the virus gets entry to the basal layer of the epithelium is adequate to neutralize the virus. The series of conformal changes that the virus needs to undergo before being able to bind to the cell surface receptors and enter the basal cells of the epithelium takes several hours. This gives adequate time for the antibody exuded at the site of infection to neutralize the virus and the phagocytes attracted to the site of trauma to opsonize the antigen–antibody complex [21]. Adequate protection offered by low antibody levels has been demonstrated in animal studies [22].

We observed a higher percentage of women with detectable antibodies to HPV6 and 18 among unvaccinated and at baseline

Table 3
Distribution and comparison of geometric mean neutralization titres for vaccine-targeted HPV types 16, 18, 6 and 11 obtained using PBNA assay by dose received and time of sample collection.

HPV type / Vaccine dose received	No. of samples	No. of samples with detectable neutralization GMT (%)*		Neutralization GMT (95 % CI)**			Neutralization GMT ratio (95 % CI) Alternate/3-dose$			Neutralization GMT ratio (95 % CI) Dose received/Unvaccinated		
HPV 16												
Unvaccinated cohort	340	24	(7.1)	383	(219 -	670)				1.00		
Day 1												
Vaccinated cohorts	170	5	(2.9)	52	(41 -	66)				0.14	(0.04 -	0.47)
Month 7												
3-dose (days 1, 60 and 180)	120	120	(100.0)	42,248	(36,280 -	49,198)				110.26	(72.85 -	166.87)
Month 18												
Single-dose	58	56	(96.6)	558	(416 -	750)	0.06	(0.04 -	0.09)	1.46	(0.82 -	2.57)
2-dose (days 1 and 180)	59	59	(100.0)	9,893	(7,754 -	12,621)	1.00	(0.67 -	1.49)	25.82	(15.49 -	43.02)
3-dose (days 1, 60 and 180)	60	60	(100.0)	9,906	(7,552 -	12,995)	1.00			25.85	(14.99 -	44.58)
Month 24												
Single-dose	60	58	(96.7)	575	(388 -	851)				1.50	(0.75 -	3.02)
Month 36												
2-dose (days 1 and 180)	59	59	(100.0)	5,636	(4,271 -	7,436)	0.69	(0.45 -	1.06)	14.71	(8.48 -	25.52)
3-dose (days 1, 60 and 180)	60	59	(98.3)	8,128	(6,095 -	10,839)	1.00			21.21	(12.07 -	37.29)
Month 48												
Single-dose	60	47	(78.3)	388	(284 -	530)	0.09	(0.05 -	0.15)	1.01	(0.57 -	1.81)
2-dose (days 1 and 180)	39	39	(100.0)	4,208	(3,028 -	5,848)	0.99	(0.72 -	1.35)	10.98	(6.07 -	19.86)
3-dose (days 1, 60 and 180)	34	34	(100.0)	4,261	(2,879 -	6,307)	1.00			11.12	(5.83 -	21.23)
Month 60												
Single-dose	34	32	(94.1)	440	(304 -	637)	0.10	(0.06 -	0.19)	1.15	(0.61 -	2.16)
2-dose (days 1 and 180)	179	178	(99.4)	5,425	(4,569 -	6,442)	1.25	(0.62 -	2.51)	14.16	(8.53 -	23.49)
3-dose (days 1, 60 and 180)	176	176	(100.0)	4,349	(3,577 -	5,289)	1.00			11.35	(6.45 -	19.97)
Month 120												
Single-dose	324	317	(97.8)	819	(701 -	957)	0.25	(0.18 -	0.35)	2.14	(1.19 -	3.84)
2-dose (days 1 and 180)	190	189	(99.5)	3,681	(3,079 -	4,401)	1.14	(0.83 -	1.55)	9.61	(5.62 -	16.41)
3-dose (days 1, 60 and 180)	167	167	(100.0)	3,237	(2,611 -	4,012)	1.00			8.45	(4.63 -	15.41)
HPV 18												
Unvaccinated cohort	340	11	(3.2)	84	(53 -	135)				1.00		
Day 1												
Vaccinated cohorts	170	1	(0.6)	58	(0 -	0)				0.69	(0.13 -	3.52)
Month 7												
3-dose (days 1, 60 and 180)	120	120	(100.0)	8,353	(6,832 -	10,212)				99.26	(50.46 -	195.26)
Month 18												
Single-dose	58	39	(67.2)	156	(113 -	216)	0.08	(0.05 -	0.14)	1.86	(0.97 -	3.56)
2-dose (days 1 and 180)	59	59	(100.0)	819	(573 -	1,170)	0.42	(0.27 -	0.65)	9.73	(4.17 -	22.72)
3-dose (days 1, 60 and 180)	60	59	(98.3)	1,951	(1,403 -	2,713)	1.00			23.18	(10.57 -	50.86)
Month 24												
Single-dose	60	41	(68.3)	191	(119 -	309)				2.27	(0.88 -	5.87)
Month 36												
2-dose (days 1 and 180)	59	56	(94.9)	566	(405 -	790)	0.37	(0.24 -	0.57)	6.72	(3.09 -	14.61)
3-dose (days 1, 60 and 180)	60	58	(96.7)	1,528	(1,101 -	2,122)	1.00			18.16	(8.35 -	39.46)
Month 48												
Single-dose	60	30	(50.0)	105	(73 -	151)	0.15	(0.08 -	0.28)	1.24	(0.65 -	2.39)
2-dose (days 1 and 180)	39	39	(100.0)	398	(265 -	597)	0.56	(0.31 -	1.00)	4.72	(2.13 -	10.49)
3-dose (days 1, 60 and 180)	34	33	(97.1)	710	(461 -	1,094)	1.00			8.44	(3.84 -	18.51)
Month 60												
Single-dose	34	17	(50.0)	162	(91 -	289)	0.29	(0.20 -	0.42)	1.93	(0.89 -	4.21)
2-dose (days 1 and 180)	179	171	(95.5)	453	(380 -	540)	0.82	(0.42 -	1.60)	5.39	(2.67 -	10.86)
3-dose (days 1, 60 and 180)	176	166	(94.3)	552	(451 -	677)	1.00			6.56	(2.95 -	14.62)
Month 120												
Single-dose	324	159	(49.1)	172	(146 -	204)	0.67	(0.49 -	0.92)	2.05	(1.08 -	3.90)
2-dose (days 1 and 180)	190	156	(82.1)	253	(213 -	301)	0.99	(0.76 -	1.28)	3.01	(1.56 -	5.81)
3-dose (days 1, 60 and 180)	167	141	(84.4)	256	(210 -	312)	1.00			3.04	(1.48 -	6.27)
HPV 6												
Unvaccinated cohort	340	22	(6.5)	313	(218 -	449)				1.00		
Day 1												
Vaccinated cohorts	170	7	(4.1)	245	(156 -	384)				0.78	(0.40 -	1.53)
Month 7												
3-dose (days 1, 60 and 180)	120	120	(100.0)	50,409	(43,214 -	58,802)				161.15	(109.32 -	237.55)
Month 18												
Single-dose	58	52	(89.7)	960	(749 -	1,231)	0.07	(0.05 -	0.10)	3.07	(1.97 -	4.77)
2-dose (days 1 and 180)	59	59	(100.0)	10,867	(7,908 -	14,934)	0.78	(0.59 -	1.03)	34.74	(19.85 -	60.82)
3-dose (days 1, 60 and 180)	60	60	(100.0)	13,936	(9,623 -	20,181)	1.00			44.55	(23.38 -	84.88)
Month 24												
Single-dose	60	54	(90.0)	1,083	(750 -	1,565)				3.46	(1.87 -	6.40)
Month 36												
2-dose (days 1 and 180)	59	59	(100.0)	6,586	(4,729 -	9,171)	0.52	(0.37 -	0.73)	21.05	(11.79 -	37.61)
3-dose (days 1, 60 and 180)	60	59	(98.3)	12,553	(8,916 -	17,673)	1.00			40.13	(22.09 -	72.89)

(*continued on next page*)

Table 3 (*continued*)

HPV type / Vaccine dose received	No. of samples	No. of samples with detectable neutralization GMT (%)*		Neutralization GMT (95 % CI)**			Neutralization GMT ratio (95 % CI) Alternate/3-dose$			Neutralization GMT ratio (95 % CI) Dose received/Unvaccinated		
	Month 48											
Single-dose	60	38	(63.3)	461	(360 -	591)	0.13	(0.09 -	0.19)	1.48	(0.97 -	2.24)
2-dose (days 1 and 180)	39	39	(100.0)	3,270	(2,265 -	4,721)	0.91	(0.83 -	0.99)	10.45	(6.03 -	18.12)
3-dose (days 1, 60 and 180)	34	34	(100.0)	3,611	(2,263 -	5,763)	1.00			11.55	(6.10 -	21.86)
	Month 60											
Single-dose	34	27	(79.4)	882	(605 -	1,286)	0.26	(0.12 -	0.56)	2.82	(1.68 -	4.73)
2-dose (days 1 and 180)	179	176	(98.3)	3,262	(2,723 -	3,907)	0.96	(0.52 -	1.80)	10.43	(6.17 -	17.62)
3-dose (days 1, 60 and 180)	176	171	(97.2)	3,381	(2,773 -	4,123)	1.00			10.81	(6.13 -	19.05)
	Month 120											
Single-dose	324	260	(80.2)	745	(655 -	848)	0.27	(0.21 -	0.35)	2.38	(1.51 -	3.75)
2-dose (days 1 and 180)	190	181	(95.3)	2,054	(1,728 -	2,442)	0.75	(0.56 -	1.01)	6.57	(3.94 -	10.94)
3-dose (days 1, 60 and 180)	167	154	(92.2)	2,727	(2,237 -	3,324)	1.00			8.72	(5.08 -	14.96)
HPV 11												
Unvaccinated cohort	340	36	(10.6)	242	(210 -	279)				1.00		
	Day 1											
Vaccinated cohorts	170	1	(0.6)	163	(0 -	0)				0.67	(0.29 -	1.58)
	Month 60											
Single-dose	34	23	(67.6)	509	(344 -	753)	0.26	(0.17 -	0.39)	2.10	(1.48 -	2.97)
2-dose (days 1 and 180)	179	171	(95.5)	2,906	(2,495 -	3,384)	1.48	(0.74 -	2.99)	12.00	(8.56 -	16.83)
3-dose (days 1, 60 and 180)	176	164	(93.2)	1,957	(1,629 -	2,352)	1.00			8.08	(5.43 -	12.03)
	Month 120											
Single-dose	324	232	(71.6)	639	(563 -	725)	0.50	(0.41 -	0.61)	2.64	(1.91 -	3.65)
2-dose (days 1 and 180)	190	181	(95.3)	1,438	(1,242 -	1,664)	1.14	(0.86 -	1.49)	5.94	(4.25 -	8.29)
3-dose (days 1, 60 and 180)	167	151	(90.4)	1,266	(1,079 -	1,485)	1.00			5.23	(3.74 -	7.30)

GMT: Geometric mean titres; CI: confidence interval; HPV. human papilloma virus; * the cut-off for the lowest detectable titres were 40 for HPV 16 and HPV 18, 155 for HPV 6 and 156 for HPV 11. ** The HPV type-specific mean antibodies were calculated using data of participants with detectable antibodies; $ Other dose schedules were non-inferior to the 3-dose schedule if the lower bound of the 95 % CI for the MFI ratio was above 0.5 (2-fold difference)

prior to vaccination compared to HPV16 and 11. Given the lack of true seropositivity threshold for HPV antibodies, this could be attributed to the low limits of quantitation for these types and possible detection of cross-reactive antibodies/binding to non-neutralizing epitopes rather than true infection.

Boosting of vaccine responses by natural infection is likely to be uncommon, since the virion antigen load transferred from an infected partner in the genital tract is usually too low to induce an anamnestic response [23]. However, we observed a rising trend in the levels of antibodies against HPV 16 and HPV 18 around 120 months in the single dose recipients, which needs further evaluation. The participants of our single dose cohort were unmarried at the time of vaccination. Many of them got married around 120 months and could be naturally infected, which could have some boosting effect. The small impact of natural booster may not be perceptible in the two or three dose arms due to already existing high antibody titre.

5. Conclusions

By using two independent technologies to assess antibody response performed at two different laboratories in a blinded manner our study demonstrates that the immunogenicity of a single dose remains high even a decade after vaccination. The trends in the antibody kinetic curves show that the titres are very unlikely to wane in another five to ten years beyond ten years post-vaccination. The laboratory data correlate very well with the clinical outcomes showing high and durable protection of a single dose against persistent infection as well as high grade cervical precancer lesions. The very recently released data from the KEN-SHE randomized controlled trial showing single-dose vaccine efficacy against incident persistent HPV 16/18 infection exceeding 90 % will make the evidence even more compelling [9].

PB has received research funding from GlaxoSmithKline through Chittaranjan National Cancer Institute, India during his previous position at the institute. NB has received research funding through her institute from GlaxoSmithKline and Merck. SJ has received funds from GlaxoSmithKline through the Jehangir Clinical Development Center for an HPV vaccine study. TW serves on advisory boards for MSD (Merck) Sharp & Dohme. The other authors declare no competing interests.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are very grateful to the Bill & Melinda Gates Foundation for their generous financial port, European Commission Seventh Framework Programme grant HPV-AHEAD (FP7-HEALTH-2011-282562) for partial support for the establishment of the Luminex-based assays at Rajiv Gandhi Centre for Biotechnology (RGCB), Trivandrum, India; Peter Dull and Carolyn Wendell (Integrated Clinical Vaccine Development, Bill & Melinda Gates Foundation) for their valuable support, encouragement, and scientific inputs; current and past members of the data safety monitoring board: Lynette Denny, John Schiller, Peter Sasieni, Thangarajan Rajkumar, Doreen Ramogola-Masire, Lutz Gissmann and Raul Murillo for their valuable advice and monitoring of the study safety and outcomes; Union for International Cancer Control for the award of International Cancer Technology Transfer fellowships that helped technology transfer and quality assurance for immunogenicity and HPV genotyping studies at the RGCB. This work was partially

supported by the DBT/Wellcome Trust India Alliance Fellowship [grant number IA/CPHI/17/1/503366] awarded to Devasena Anantharaman. We thank Mrs Krittika Guinot, IARC, for help in preparation of the manuscript.

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