Vaccine 36 (2018) 98–106

Contents lists available at ScienceDirect

Vaccine

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

Comparative immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and 4vHPV vaccine administered according to two- or three-dose schedules in girls aged 9–14 years: Results to month 36 from a randomized trial

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ARTICLE INFO

Article history:
Received 26 April 2017
Received in revised form 26 July 2017
Accepted 13 November 2017
Available online 23 November 2017

Keywords:
Human papillomavirus vaccines
Administration schedule
Female adolescents
Immunogenicity
Safety

ABSTRACT

This observer-blind study (clinicaltrials.gov NCT01462357) compared the immunogenicity and safety of two doses (2D) of the HPV-16/18 AS04-adjuvanted vaccine (2D of AS04-HPV-16/18) vs. two or three doses of the 4vHPV vaccine [2D or 3D of 4vHPV] in 1075 healthy girls aged 9–14 years. Girls were randomized (1:1:1) to receive 2D of AS04-HPV-16/18 at months (M) 0, 6 (N = 359), 2D of 4vHPV at M0, 6 (N = 358) or 3D of 4vHPV at M0, 2, 6 (N = 358). 351, 339 and 346 girls, respectively, returned for the concluding visit at M36. Superiority was demonstrated at M7 and M12; comparison of the immune response to both vaccine antigens was made between 2D of AS04-HPV-16/18 and 2D or 3D of 4vHPV at subsequent time points in the according-to-protocol immunogenicity cohort (ATP-I; N = 958 at M36) and the total vaccinated cohort (TVC: N = 1036 at M36). HPV-16/18-specific T-cell- and B-cell-mediated immune responses and safety were also investigated. At M36, anti-HPV-16/18 ELISA responses in the 2D AS04-HPV-16/18 group remained superior to those of the 2D and 3D 4vHPV groups. In the M36 TVC, geometric mean titers were 2.78-fold (HPV-16) and 6.84-fold (HPV-18) higher for 2D of AS04-HPV-16/18 vs. 2D of 4vHPV and 2.3-fold (HPV-16) and 4.14-fold (HPV-18) higher vs. 3D of 4vHPV. Results were confirmed by vaccine pseudovirion-based neutralisation assay. Numbers of circulating $CD4^+$ T cells and B cells appeared similar across groups. Safety was in line with the known safety profiles of both vaccines. In con-

Abbreviations: 2D, 2-dose; 3D, 3-dose; AAHS, aluminum hydroxyphosphate sulphate; AE, adverse event; ANOVA, analysis of variance; AS04, Adjuvant System containing 50 μg 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL) adsorbed on aluminum salt (500 μg Al^{3+}); ATP, according-to-protocol; CI, confidence interval; CMI, cell-mediated immunity; ED_{50}, effective dose producing 50% response; ELISA, enzyme-linked immunosorbent assay; EU, ELISA unit; GMR, geometric mean titer ratio; GMT, geometric mean antibody titer; HPV, human papillomavirus; AS04-HPV-16/18, HPV-16/18 AS04-adjuvanted vaccine; 4vHPV, HPV6/11/16/18 vaccine; IFNγ, interferon-gamma; IL, Interleukin; M, month(s); IgG, immunoglobulin G; PBMC, peripheral blood mononuclear cells; PBNA, pseudovirion-based neutralisation assay; pIMD, potential immune-mediated disease; SAE, serious adverse event; TNFα, tumor necrosis factor alpha; TVC, total vaccinated cohort; VLP, virus-like particle; y, year(s).

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https://doi.org/10.1016/j.vaccine.2017.11.034
0264-410X/

clusion, superior HPV-16/18 antibody responses were elicited by 2D of the AS04-HPV-16/18 compared with 2D or 3D of the 4vHPV vaccine in girls aged 9–14 years.
Clinical Trial Registration: NCT0146235.

1. Introduction

Today's estimates suggest that over 500,000 women are diagnosed with cervical cancer every year(y) and more than 260,000 die from the disease [1]. Cervical cancer is one of the most frequent cancers in women throughout the World [1,2]. Human papillomavirus (HPV)-16 and HPV-18 are responsible for approximately 70% of cervical cancer cases [3–7].

HPV vaccination began in 2006/2007 [8–10] when the first two HPV vaccines, the HPV-16/18 AS04-adjuvanted (AS04-HPV-16/18) vaccine (*Cervarix*, GSK) and the HPV6/11/16/18 (4vHPV) vaccine (*Gardasil*, Merck & Co., Inc.) were licensed for the prevention of cervical cancer and high-grade precursor lesions. Both vaccines contain L1 virus-like particles (VLPs) from the two oncogenic HPV types most prevalent in cervical cancer, i.e. HPV-16 and HPV-18 [1]. The main differences in the composition of both vaccines are the inclusion of HPV-6 and -11 L1 VLPs in the 4vHPV vaccine and the AS04 adjuvantation in the HPV-16/18 vaccine. In addition, the VLPs are manufactured by different methods [1]. More recently, a nonvalent vaccine was licensed using the same HPV-6, -11, -16 and -18 antigens as the four-valent vaccine and VLPs for 5 additional oncogenic HPV types (*Gardasil 9*, Merck & Co. Inc).

The initially licensed schedule for the HPV vaccines comprised 3 doses administered at months (M) 0, 1 or 2, and 6 [7–13]. However, high vaccine coverage and compliance rates proved to be difficult to achieve with a 3-dose (3D) regimen. The high immune response to the AS04-HPV-16/18 vaccine observed in the adolescent population 9-14y of age led to the investigation and eventually to registration of 2-dose schedules (2D) in this age group in most countries [14,15]. WHO started recommending a 2D in young girls from 2014 [16].

The mechanism of protection against mucosal infection is essentially thought to be antibody-mediated. Superiority in terms of neutralizing antibody titers after vaccination with Cervarix compared to Gardasil was previously demonstrated in adult women with the standard 3D schedule [17–21]. Higher anti-HPV antibody titers have the potential to elicit a longer duration of protection. The comparison of the immunogenicity elicited by the reduced schedule of Cervarix compared to both 2D and 3D of Gardasil vaccines was therefore warranted in the HPV naïve population targeted by mass vaccination programs where the duration of protection is of paramount importance.

This study was thus designed to assess immunogenicity and safety of a 2D schedule of the AS04-HPV-16/18 vaccine vs. 2D and 3D of the 4vHPV vaccine in girls aged 9-14y.

2. Material and methods

2.1. Study design and ethics

The study was observer-blind, randomized and age-stratified with three parallel groups (Fig. 1) conducted at 21 sites in France, Hong Kong, Singapore and Sweden (November 2011 to October 2015). The trial is registered with ClinicalTrials.gov (NCT01462357) and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Protocol is summarized at www.gsk-clinicalstudyregister.com (GSK Study Identifier 115411).

The primary objective was to evaluate if immunogenicity to HPV-16 and -18, as measured by enzyme-linked immunosorbent assay (ELISA), of a 2D AS04-HPV-16/18 vaccine was non-inferior/superior to a 2D 4vHPV vaccine 1 M after the last vaccine dose (M7). Secondary objectives included evaluation of the non-inferiority/superiority of 2D AS04-HPV-16/18 vs. 2D and 3D 4vHPV at all subsequent time points until study conclusion (M36). Other secondary immunogenicity objectives included assessment of HPV-16/-18 neutralizing antibodies (by pseudovirion-based neutralisation assay (PBNA)) and frequencies of specific memory B and T cells. Safety was also evaluated.

2.2. Study participants

Healthy girls aged 9-14y were eligible to participate as per the protocol requirements. Informed consent was obtained from subjects and their parents or legal guardians.

2.3. Vaccines, randomization and masking

Enrolment was stratified by age (approximately 50% aged 9-11y and 50% aged 12-14y), and girls were randomized (1:1:1 ratio in each age stratum) to receive either 2D AS04-HPV-16/18 (at M0,6, 2D HPV-6/11/16/18 at M0,6 or 3D HPV-6/11/16/18 at M0,2,6, in the deltoid muscle of the non-dominant arm. Compositions of both vaccines have been described previously [1]. Batch numbers of vaccine lots were AHPVA144B, AHPVA133C, AHPVA133E, AHPVA151C, AHPVA133A and AHPVA184C for AS04-HPV-16/18; NP39130, and H006966 for 4vHPV; and PHPVA012A for placebo vaccine.

The study was observer-blind. Girls from the 2D groups received placebo [Al $(OH)_3$] at M2 to maintain the blinding. The randomization code was generated using *MATEX*, a program developed for use in *SAS* (Cary, NC, USA), by GSK, Belgium.

In pre-selected sites, the first 50 subjects from each age stratum in each group (300) were assigned to the cell-mediated immunity (CMI) sub-cohort for measurement of circulating HPV-specific B- and T-lymphocytes. The same subjects were included in the PBNA subset.

2.4. Immunogenicity assessments

Blood was sampled at M0 (pre-vaccination) and at M7, 12, 18, 24 and 36 for the measurement of HPV-16/-18 antibodies by ELISA and PBNA in a subset. An additional blood sample was taken from girls assigned to the subset.

Anti-HPV-16/-18 antibodies were determined by ELISA using the purified type-specific recombinant VLPs present in the AS04-HPV-16/18 vaccine as coating antigen [7,22]. Seronegativity corresponded to a titer lower than assay cut-off (19 ELISA units [EU]/mL for anti-HPV-16 and 18 EU/mL for anti-HPV-18). Neutralizing HPV-16/-18 antibodies were determined by PBNA [1,23]. Pseudovirions were produced independent of vaccine constructs as described previously [22]. In this procedure, assay cut-off is 40 ED_{50} (effective dose producing 50% response, for each antigen).

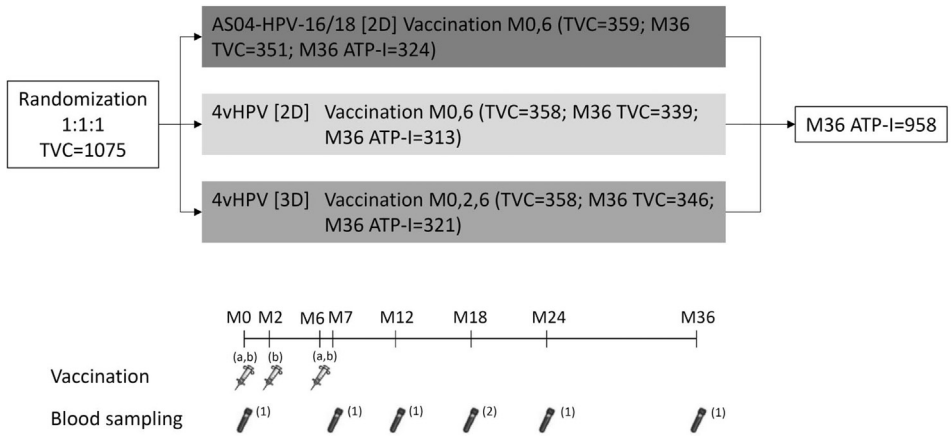

Fig. 1. Study design and flow of participants through the trial up to Month 36. Syringe symbols represent vaccine administration in [a]2-doses (2D) and [b]3-doses (3D) schedule of AS04-HPV-16/18 or HPV-6/11/16/18 (4vHPV). vaccine; Blood samples were collected [1]for assessment of antibodies (by enzyme-linked immunosorbent assay in all subjects and by pseudovirion-based neutralisation assay in a subset of subjects); and [2]for assessment of cell-mediated immunity in a subcohort of subjects; M, Month; ATP-I, According-to-protocol cohort for immunogenicity; TVC, total vaccinated cohort.

The frequencies of HPV-specific memory B-lymphocytes were measured by using standard enzyme-linked immunosorbent spot assay [24]. Briefly, peripheral blood mononuclear cells (PBMC) from clinical trial subjects were stimulated with oligonucleotide containing Cytosine phosphate Guanine motives during 5 days to induce differentiation into antibody secreting cells. Polyvinylidene difluoride plates were coated with either an antibody specific for human immunoglobulins) or with L1 VLP antigens present in the AS04-HPV-16/18 vaccine [1,25] to quantify total or antigen specific memory B-cells, respectively. Activated memory B-cells were then incubated overnight on plate, and spot forming cells were detected on day 6 by the addition of secondary antibody, conjugate complex and substrate. Finally, plates were dried overnight before analysis using an automated counting spot system (Axiovision).

The frequencies of HPV-16 and -18-specific CD4+ and CD8+ T-lymphocytes were evaluated using a standard Intracellular Cytokine Staining assay. Briefly, PBMC from clinical trial subjects were stimulated overnight with a pool of peptides covering the entire sequence of L1 antigens from HPV16/18. Cells were then stained with CD3, CD4 and CD8 antibodies, fixed and permeabilized, and further stained with antibodies specific for immune markers [CD40L, interleukin (IL) 2, tumor necrosis factor (TNFα) and interferon-gamma (IFNγ)]. Flow cytometry was then used to quantify number of lymphocytes producing at least 2 out of the 4 different immune markers assessed [17,26–28].

2.5. Reactogenicity and safety

Reactogenicity and safety findings up to M12 were reported in a previous paper [29]. It presents the data for serious adverse events (SAEs) and medically significant adverse events (AEs) up to M36.

2.6. Statistical methods

The hierarchy of testing for immunological non-inferiority and superiority comparisons was pre-specified in the protocol. The most conservative dataset was chosen for each analysis [30]. For superiority testing, the total vaccinated cohort (TVC) (with at least one documented vaccine dose), was used as the primary analysis set because it tends to avoid over-optimistic estimates of efficacy resulting from a per protocol analysis, since the non-compliers included in the full analysis set will generally diminish the estimated treatment effect. However, in an equivalence or non-inferiority trial use of the full analysis set is generally not conservative and its role should be considered very carefully. A more stringent according-to-protocol (ATP) analysis set was used for the primary analysis of non-inferiority comparisons. The ATP included subjects who received planned vaccine doses, met all eligibility criteria, complied with all requirements and for whom immunogenicity data were available. Non-inferiority in terms of seroconversion was shown if, for both anti-HPV-16 and -18, the upper limits of the 95% confidence intervals (CI) for the differences (2D or 3D 4vHPV minus 2D AS04-HPV-16/18 vaccine) were below 5%. Non-inferiority in terms of geometric mean antibody titers (GMT) was shown if, for both, the upper limits of the 95% CIs for the GMT ratios (2D or 3D 4vHPV divided by 2D AS04-HPV-16/18 vaccine) were below 2. If non-inferiority was demonstrated and lower limits of the two-sided 95% CIs for the ratio of GMTs of a given antigen were above 1 in the ATP, superiority was assessed sequentially in the TVC. Superiority was demonstrated if lower limit of the 95% CI for the ratio of GMTs (2D AS04-HPV-16/18 vaccine divided by 2D or 3D 4vHPV) was above 1. Objectives were assessed sequentially. For the non-inferiority analysis, 285 evaluable subjects/group in ATP would allow the detection of a 5% difference in anti-HPV-16/-18 seroconversion rates 1 M after the last dose with 95% power and the detection of a 2-fold difference in anti-HPV-16/-18 GMTs with at least 99% power. A sample size of 322 subjects/group in TVC would allow the demonstration of superiority in terms of GMTs 1 M after the last dose with at least 99% power. Assuming that 20% of subjects would withdraw or would not be evaluable for immunogenicity 1 M after the last dose, the target sample size for enrolment was 1074 subjects (358/group).

Seroconversion, seropositivity rates and GMTs for HPV-16/-18 antibodies were calculated by baseline serostatus. Frequencies of HPV-16 and -18-specific memory B- and T-lymphocytes at each time point were summarized for each group using descriptive statistics. Immunogenicity analyses focused on subjects who were seronegative at baseline. Supplementary analyses were done by baseline serostatus and based on the TVC. Descriptive comparisons were made between anti-HPV-16/-18 GMTs in this trial and historical data, i.e. in women aged 15-25y (by ELISA) or 18-45y (by PBNA) who had cleared a natural infection and mounted an immune response [1,31] and GMTs (by ELISA) from the plateau phase (M45-50) of a long-term efficacy study in 15-to-25-year-old women vaccinated with AS04-HPV-16/18 [8]. The plateau phase observed in the efficacy studies HPV-001/007/023 corresponds to GMTs in subjects for which vaccine efficacy was

Table 1
Demographic characteristics and baseline serostatus in the TVC and ATP at M36.

	2D of AS04-HPV-16/18	2D of 4vHPV	3D of 4vHPV
Demographic characteristics			
TVC	**N = 359**	**N = 358**	**N = 358**
Age (years) at time of first vaccine dose, mean (SD)	11.5 (1.64)	11.5 (1.56)	11.6 (1.64)
Geographic ancestry, n (%)			
Asian Heritage	261 (72.7)	257 (71.8)	264 (73.7)
White Heritage	94 (26.2)	93 (26.0)	90 (25.1)
African Heritage/African American	4 (1.1)	6 (1.7)	4 (1.1)
Other	0 (0.0)	2 (0.6)	0 (0.0)
Baseline serology			
TVC	**N = 359**	**N = 358**	**N = 358**
HPV-16 baseline serostatus by ELISA, n (%)			
Seronegative	352 (98.1)	349 (97.5)	345 (96.4)
Seropositive	7 (1.9)	9 (2.5)	13 (3.6)
HPV-18 baseline serostatus by ELISA, n (%)			
Seronegative	356 (99.2)	355 (99.2)	357 (99.7)
Seropositive	3 (0.8)	3 (0.8)	1 (0.3)
ATP at M36	**N = 324**	**N = 313**	**N = 321**
HPV-16 baseline serostatus by ELISA, n (%)			
Seronegative	318 (98.1)	306 (97.7)	309 (96.3)
Seropositive	6 (1.9)	7 (2.3)	12 (3.7)
HPV-18 baseline serostatus by ELISA, n (%)			
Seronegative	322 (99.4)	310 (99.0)	320 (99.6)
Seropositive	2 (0.6)	3 (1.0)	1 (0.4)

ATP-I, according-to-protocol immunogenicity cohort; ELISA, enzyme-linked immunosorbent assay; 2-doses (2D) and 3-doses (3D) schedule of AS04-HPV-16/18 or HPV-6/11/16/18 (4vHPV) vaccine; N, total number of subjects; n (%), number (percentage) of subjects in a given category; SD, standard deviation; TVC, total vaccinated cohort. Seronegative status defined as an antibody titer lower than the assay cut-off (19 ELISA units [EU]/mL for anti-HPV-16 and 18 EU/mL for anti-HPV-18).

demonstrated. The plateau level is not used as a correlate of protection but is informative and serves as a benchmark for studies with AS04-HPV-16/18 vaccine. The plateau level is not used for clinical management of subjects.

Safety data were summarized descriptively in the TVC. Statistical testing was not planned or conducted for safety and inferences were based on descriptive comparisons. Statistical analyses were performed using *SAS* 9.2 and PROC *StatXact* 8.1.

3. Results

3.1. Study population

A total of 1075 girls aged 9-14y received at least one vaccine dose (359 for 2D AS04-HPV-16/18, 358 for 2D 4vHPV, 358 for 3D 4vHPV) and 1036 (96%) completed the study to M36 (Fig. 1). Overall, 958 (89%) girls were included in the ATP cohort for immunogenicity at M36. Compliance with the planned vaccination schedule was high (≥95% in each group). All groups were well matched with regard to demographic data (Table 1). For all cohorts, the majority of girls in each group were seronegative for anti-HPV-16 and anti-HPV-18 at baseline (Table 1).

3.2. Immunological non-inferiority and superiority

The primary objective and results up to M12 were described previously [24]. Non-inferiority/superiority was also successfully demonstrated up to M36. For the 2D AS04-HPV-16/18 vs. 2D 4vHPV comparisons, superiority was shown in terms of GMT ratios, confirming that the 2D AS04-HPV-16/18 elicited antibody titers at M36 that were more than two and sixfold higher for anti-HPV-16 (2.78) and anti-HPV-18 (6.84), respectively (Table 2). Similar results were shown for the comparison of 2D AS04-HPV-16/18 and 3D 4vHPV with antibody titers that were more than two and fourfold higher for anti-HPV-16 (2.30) and anti-HPV-18 (4.14), respectively, with AS04-HPV-16/18 (Table 2).

3.3. Antibody responses

In each group, all initially seronegative subjects from the ATP cohort had seroconverted for HPV-16/-18 antibodies at M7 when measured by ELISA and PBNA. At M36, all initially seronegative subjects (100%) from the 2D AS04-HPV-16/18 group and nearly all subjects from the 2D (99.3%) and 3D (99.7%) 4vHPV groups had seroconverted for anti-HPV-16 antibodies when measured by ELISA. For HPV-18 antibodies, all subjects (100%) from the 2D AS04-HPV-16/18 group were still seroconverted at M36. In the 4vHPV 2D and 3D groups, a proportion of subjects became seronegative during the course of the study, i.e. 13.9% with 2D 4vHPV and 7.2% with 3D 4vHPV.

Similar results were obtained by PBNA in the M36 ATP cohort for immunogenicity since all initially seronegative subjects (100%) from the 2D AS04-HPV-16/18 and 2D or 3D 4vHPV groups were still seroconverted for anti-HPV-16 neutralizing antibodies at study end. For HPV-18, all initially seronegative subjects (100%) from the AS04-HPV-16/18 group were still seroconverted at study end, while 13.8% of subjects in the 2D 4vHPV group and 5.5% of subjects in the 3D 4vHPV group had antibody titers below the assay cut-off at M36.

HPV-16/18 GMTs, which had reached a peak response at M7, gradually declined up to M36 in all three groups. Titers remained above the plateau for HPV-16 in all groups, but fell below the threshold observed in *Cervarix* studies for HPV-18 antibodies in both 4vHPV groups (Fig. 2). HPV-18 antibodies fell below the plateau at M12 with the 2D regimen and, by M18, with the 3D 4vHPV. Antibody titers with the 2D AS04-HPV-16/18 remained well above the plateau for both antigens. Similar results were observed at M36 in the TVC regardless of the baseline serostatus (Table 2 C).

Neutralizing antibody titers were found to be more than two and sixfold higher for HPV-16 and HPV-18 in the AS04-HPV-16/18 vaccine group vs. the 4vHPV groups, respectively.

3.4. Cell-mediated immunity

HPV-16/18-specific memory B cell- and $CD4^+$ T cell-mediated responses were similar across all three groups (Fig. 3).

Table 2
Non-inferiority (A) and superiority (B) of anti-HPV-16/18 responses and Immunogenicity (C) results of 2D of AS04-HPV-16/18 vaccine, 2D and 3D of 4vHPV at M36.

Comparison	A. Non-inferiority (ATP-I, initially seronegative subjects)		B. Superiority (TVC)
Antibody	SCR difference % (95% CI)	GMT ratio (95% CI)	GMT ratio (95% CI)
AS04-HPV-16/18[2D] vs. 4vHPV [2D]	4vHPV[2D] minus AS04-HPV-16/18 [2D]	Ratio of AS04-HPV-16/18 [2D] to 4vHPV [2D]	Ratio of AS04-HPV-16/18[2D] to 4vHPV [2D]
Anti-HPV-16	-0.65 (-2.35; **0.55**)	0.36 (0.31; **0.42**)	2.78 (**2.38** – 3.24)
Anti-HPV-18	-13.87 (-18.17; **-10.46**)	0.15 (0.12; **0.17**)	6.84 (**5.81** – 8.05)
AS04-HPV-16/18[2D] vs. 4vHPV [3D]	4vHPV[3D] minus AS04-HPV-16/18 [2D]	Ratio of 4vHPV[3D] to AS04-HPV-16/18 [2D]	Ratio of AS04-HPV-16/18[2D] to 4vHPV [3D]
Anti-HPV-16	-0.32 (-1.81; **0.87**)	0.45 (0.39; **0.51**)	2.30 (**2.00** – 2.64)
Anti-HPV-18	-7.19 (-10.56; **-4.84**)	0.24 (0.21; **0.29**)	4.14 (**3.49** – 4.91)

	C. Immunogenicity results (ATP-I, initially seronegative subjects; ELISA)					
Antibody	N	AS04-HPV-16/18[2D]	N	4vHPV[2D]	N	4vHPV[3D]
	SCR % (95% CI)					
Anti-HPV-16	318	100 (98.8; 100)	306	99.3 (97.7 – 99.9)	309	99.7 (98.2 – 100)
Anti-HPV-18	322	100 (98.9; 100)	310	86.1 (81.8 – 89.8)	320	92.8 (89.4; 95.4)
	GMT ELISA Units (95% CI)					
Anti-HPV-16	318	1061 (972 – 1159)	306	380 (333 – 433)	309	472 (425 – 525)
Anti-HPV-18	322	487 (438 – 541)	310	71 (62 – 81)	320	119 (103 – 137)
	GMT International Units (95% CI)					
Anti-HPV-16	318	174.0 (159.3 – 190.1)	306	62.3 (54.7 – 71.0)	309	77.4 (69.6 – 86.0)
Anti-HPV-18	322	85.3 (76.7 – 94.8)	310	12.5 (10.9 – 14.3)	320	20.9 (18.2 – 24.1)

Bolded values indicate that non-inferiority/superiority criteria were met. Non-inferiority in terms of seroconversion was confirmed if the upper limit of the 95% CI for the difference in seroconversion rates (4vHPV minus AS04-HPV-16/18) was less than the predefined limit of 5%. Non-inferiority in terms of GMTs was confirmed if the upper limit of the 95% CI for the GMT ratio (4vHPV divided by AS04-HPV-16/18) was below the predefined limit of 2. Superiority with respect to GMTs was confirmed if the lower limit of the 95% CI for the GMT ratio (AS04-HPV-16/18 divided by 4vHPV) was above the predefined limit of 1. ATP-I, according-to-protocol immunogenicity cohort; 2-doses (2D) and 3-doses (3D) schedule of AS04-HPV-16/18 or HPV-6/11/16/18 (4vHPV) vaccine; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; EU/mL, ELISA units per milliliter; GMT, geometric mean antibody titer; TVC, total vaccinated cohort. Seronegative status defined as an antibody titer lower than the assay cut-off at baseline (19 EU/mL for anti-HPV-16 and 18 EU/mL for anti-HPV-18).

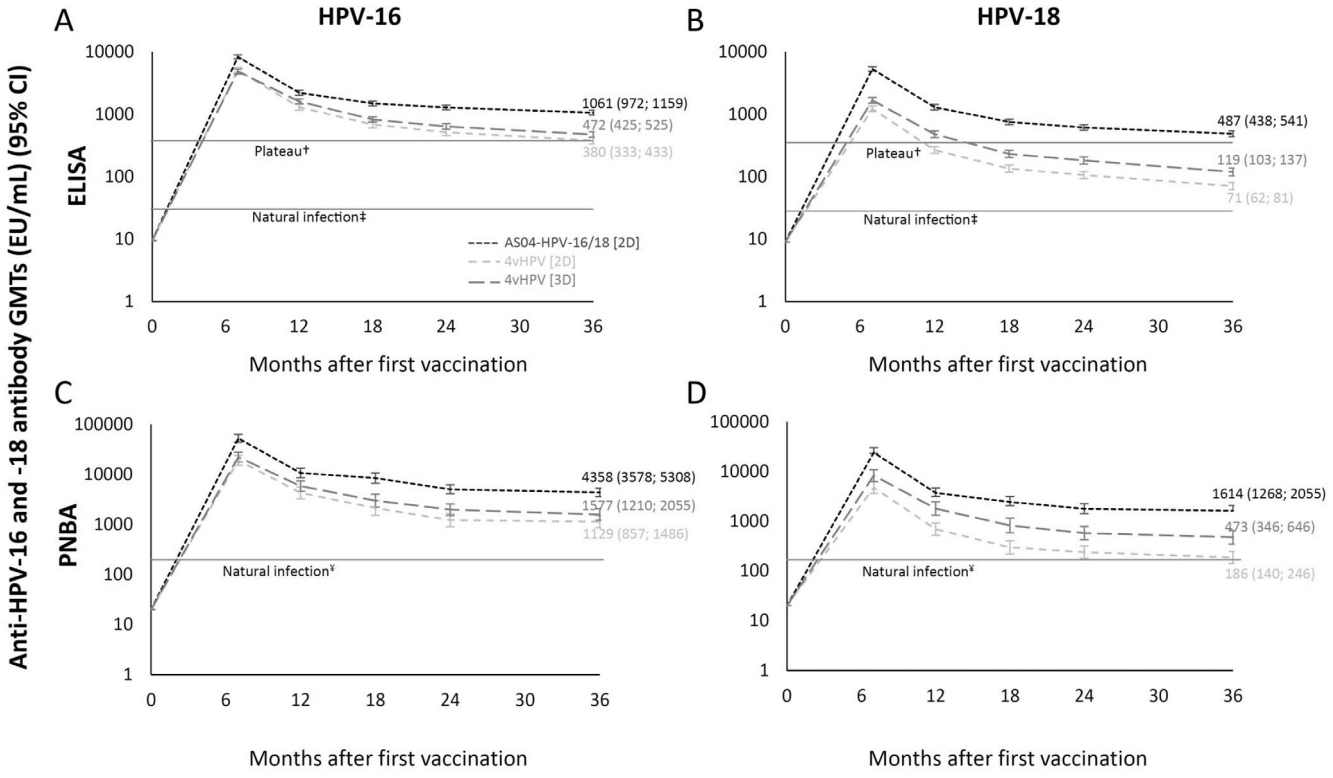

Fig. 2. Anti-HPV-16 and -18 antibody GMTs, as measured by ELISA (M36 ATP-I, initially seronegative* subjects) or PBNA (M36 ATP-I subset, initially seronegative* subjects), at Months 6, 7, 12, 18, 24 and 36 after the first vaccine dose. Plotted curves show mean GMTs (and 95% CI) by ELISA (A and B) and PNBA (ED_{50}) (C and D) for anti-HPV-16/18 for subjects who received 2-doses (2D) and 3-doses (3D) schedule of AS04-HPV-16/18 or HPV-6/11/16/18 (4vHPV) vaccine. (A and B) *The cut-off values for the ELISA assays were 19 EU/mL and 18 EU/mL for HPV-16 and -18, respectively. † GMTs (ELISA) at the plateau level (M45–50 after the first vaccine dose) in women vaccinated at the age of 15–25 years in study HPV-007 (NCT00120848) were 397.8 EU/mL for HPV-16 and 297.3 EU/mL for HPV-18; ‡ GMTs after clearing natural infection in women from study HPV-008 (NCT00122681) were 29.8 EU/mL for HPV-16 and 22.6 EU/mL for HPV-18. (C and D) *The cut-off values for the PBNA assay were 40 ED_{50}. ¥GMTs after clearing natural infection in women (aged 18–45 years) from study HPV-010 (NCT00423046) were 180.1 ED_{50} for HPV-16 and 137.3 ED_{50} for HPV-18. ATP-I, according-to-protocol cohort for immunogenicity; CI, confidence interval; ED50, effective dilution giving a 50% reduction of the signal compared to a control without serum;; ELISA, enzyme-linked immunosorbent assay; EU/mL, ELISA Units/mL; GMT, geometric mean titer; HPV, human papillomavirus; PBNA, pseudovirion-based neutralisation assay.

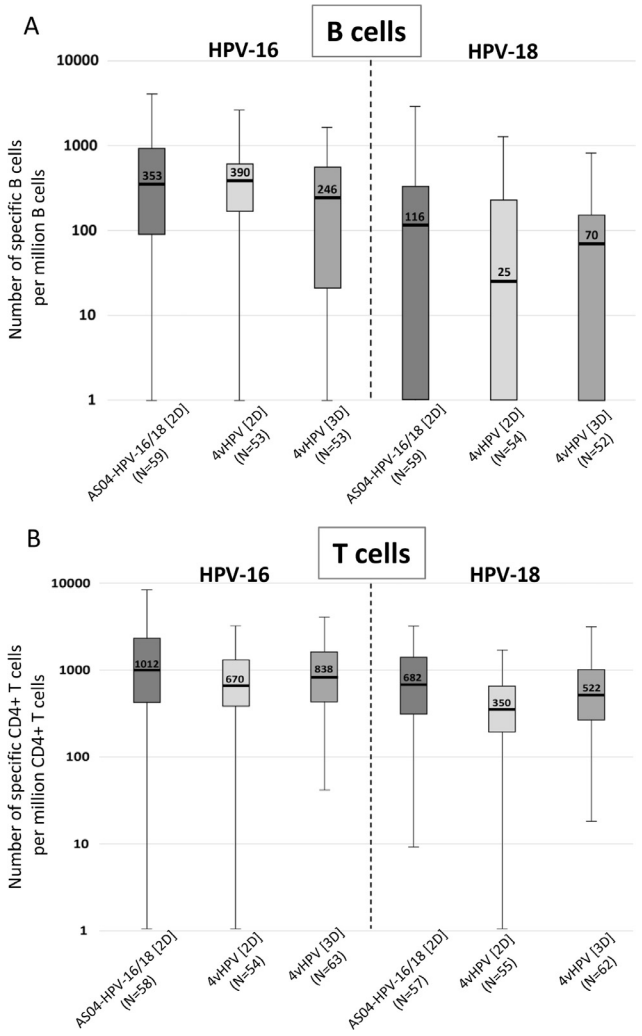

Fig. 3. Memory B-cell and CD4+ T-cell-mediated immune responses specific to HPV-16 and HPV-18 at M36 (M36 ATP-I cell-mediated immunity subset; initially seronegative subjects). 2-doses (2D) and 3-doses (3D) schedule of AS04-HPV-16/18 or HPV-6/11/16/18 (4vHPV) vaccine; ATP-I, according-to-protocol cohort for immunogenicity; HPV, human papillomavirus.

As observed at previous time points, HPV-16 and HPV-18 specific CD8+ T cells responses were undetectable (up to 4.0 cells/million CD8+ T cells) in all groups at M36. Similar results were observed in the TVC regardless of the baseline serostatus.

3.5. Safety

Solicited or unsolicited AEs and potential immune-mediated disease (pIMDs) up to M12 were reported previously [26]. Among them, only one non-serious pIMD (reactive arthritis) was considered by the investigator as possibly caused by vaccination and was related to the 4vHPV 2D group. Up to M36, a total of 46 subjects including 21 from the 2D AS04-HPV-16/18, 11 from the 2D 4vHPV and 14 from the 3D 4vHPV groups, reported at least one SAE (Table 3). One vaccine-unrelated case of fatal SAE (committed suicide) was recorded at M36 in the 3D 4vHPV group. None of the SAEs were considered by the investigator to be vaccine-related. All SAEs resolved, except ulcerative colitis with relapse episodes, food-dependent exercise induced anaphylaxis, tension headache and juvenile idiopathic arthritis events, which were on-going at the time of the M36 database lock point.

Table 3
Safety data at M36.

	AS04-HPV-16/18 [2D] (N = 359)	4vHPV [2D] (N = 358)	4vHPV [3D] (N = 358)
≥1 medically significant AE* n(%)	**77 (21.4)**	**79 (22.1)**	**63 (17.6)**
Pyrexia	5 (1.4)	7 (2.0)	5 (1.4)
Influenza	5 (1.4)	3 (0.8)	7 (2.0)
Varicella	5 (1.4)	5 (1.4)	3 (0.8)
Cough	2 (0.6)	4 (1.1)	4 (1.1)
Ligament sprain	2 (0.6)	4 (1.1)	4 (1.1)
Oropharyngeal pain	2 (0.6)	4 (1.1)	4 (1.1)
≥1 SAE n(%)**	**21 (5.8)**	**11 (3.1)**	**14 (3.9)**
Asthma	2 (0.6)	1 (0.3)	0
Gastroenteritis	3 (0.8)	0	0
Upper respiratory tract infection	2 (0.6)	0	1 (0.3)
Appendicitis	1 (0.3)	1 (0.3)	0
Foot fracture	2 (0.6)	0	0
Pneumonia	1 (0.3)	0	1 (0.3)

* Those occurring in ≥10 subjects overall are listed.
** Those occurring in ≥2 subjects overall are listed. 2-doses (2D) and 3-doses (3D) schedule of AS04-HPV-16/18 or HPV-6/11/16/18 (4vHPV) vaccine; AE, adverse event; SAE, serious adverse event.

A total of 219 subjects reported at least one Medically Significant Condition up to M36, 77 of whom were in the 2D AS04-HPV-16/18 group, 79 in the 2D 4vHPV and 63 in the 3D 4vHPV in the TVC (Table 3). In addition, one case of pregnancy, which was electively terminated with no apparent congenital anomaly, was reported in the 3D 4vHPV group.

4. Discussion

The recent licensure of 2D schedules of the HPV vaccines for preteens/adolescents is important for global public health, as this is likely to facilitate the introduction of vaccination programs in lower-income countries. This may also help improve the relatively low vaccine coverage and series completion rates observed in some higher-income countries [32–35]. This trial was undertaken to assess the immunogenicity and safety of a 2D AS04-HPV-16/18 vaccine vs. 2D and 3D 4vHPV vaccine in girls aged 9-14y. We found that anti-HPV-16/-18 GMTs, as measured by ELISA, were still significantly higher 30 M after the last vaccine dose following administration of 2D AS04-HPV-16/18 vaccine vs. 2D and 3D 4vHPV vaccine. The ELISA assay used as primary readout in this trial is based on VLPs from the AS04-HPV-16/18 vaccine. In order to exclude a potential assay-related bias in favour of the AS04-HPV-16/18 vaccine, results were also confirmed using vaccine-independent PBNA. The PBNA results were very similar to the results obtained using ELISA, as previously described, with strong correlation between ELISA and PNBA detection of anti-HPV-16/18 antibodies after vaccination [29,36].

Our findings of higher antibody responses in girls vaccinated with 2D AS04-HPV-16/18 vaccine are in agreement with previous head-to-head comparisons between the AS04-HPV-16/18 and 3D 4vHPV vaccines; and results were consistent across studies in both young girls (2/3D) and adult women (3D) [1,18,37,38]. Immunological responses were found to be approximatively 2.3–2.8 and 3.5–6.8-fold higher in AS04-HPV-16/18 compared to 4vHPV groups (2D and 3D) for HPV-16 and HPV-18, respectively. This observation from clinical trials was also made in the context of organized vaccination programs [39].

The differences in immunogenicity between both HPV vaccines may in theory be due to different production methods and/or to the adjuvants used in each vaccine; available evidence supports an important role of the adjuvant [40].

An immunological correlate of protection has not yet been defined for HPV infection and associated cervical lesions, although previous studies have shown that higher titers of naturally-acquired HPV-16 antibodies (by ELISA) and, to a lesser extent HPV-18 antibodies, were associated with lower risk of newly detected infection and cervical abnormalities caused by the same HPV type in unvaccinated subjects [41,42]. In our study, all 3 vaccine regimens induced HPV-16 and -18 antibody titers that were higher than the levels of naturally-acquired antibodies previously observed in women who had cleared a natural infection [1,31]. It has been hypothesized that this results of a better targeting or activation of lymph nodes cells by vaccines compared to mucosal infections and the use of adjuvants [16]. In those girls who received AS04-HPV-16/18, GMTs at M36 were above the plateau level of antibodies observed in women aged 15-25y participating in an efficacy trial, in whom sustained protection against HPV-16/18-associated infection and cervical lesions was shown [8]. All groups were found to have antibody levels above the plateau for HPV-16, but for HPV-18 antibodies induced by 4vHPV were below the plateau benchmark with both dose schedules (Fig. 2). The clinical relevance of this observation is not known, although the magnitude of vaccine-induced antibody titers may influence persistence of immunity.

The role of CMI in the control of HPV infections is not well established, although induction of antigen-specific memory B cells, a process in which $CD4^+$ T cells play an essential role, is thought to be important for long-term vaccine-induced protection [43–45]. Our trial was not powered to make statistical comparisons for CMI and descriptive analyses showed large overlaps in CMI responses between groups. However, the median frequencies of HPV-16 and -18-specific memory B cells and $CD4^+$ T cells at M7, M12 and M36 were numerically higher, though in the same range overall, for girls who received 2D AS04-HPV-16/18 vaccine. The enhanced $CD4^+$ T-cell response observed in the AS04-HPV-16/18 group may be related to the ability of the monophosphoryl lipid A component of the AS04 adjuvant to enhance antigen presentation to $CD4^+$ T cells, resulting in turn in increased differentiation of B cells into antibody-producing plasma cells and memory B cells [46]. Even if human studies, including ours and others [47], only show limited differences in vaccine-induced T-cell cytokine profiles between vaccines, there seem to be differences in the isotype switching of vaccine-induced antibody [48,49], which may correlate with differences in the quality of vaccine-induced adaptive cellular immunity.

Both vaccines had a clinically acceptable safety profile in this population, which is in line with the known safety profile observed in previous studies [50–52].

A strength of this descriptive study is that assessments were performed according to the same schedule and methodology in all groups, allowing a valid head-to-head comparison of immunogenicity. A limitation of the study is the absence of a virological or clinical endpoint to assess potential differences in terms of protection against HPV infections or lesions.

In summary, this study demonstrated that the 2D AS04-HPV-16/18 vaccine elicited superior antibody responses in girls aged 9 to 14y to those elicited by 2D and 3D 4vHPV vaccine up to M36. Although there is no defined correlate of protection for HPV, the higher immune response observed following vaccination with AS04-HPV-16/18 may be indicative of a longer duration of protection.

Trademarks

Cervarix is a trademark of the GSK group of companies.
Gardasil is a trademark of Merck & Co., Inc.

Acknowledgments

The authors thank the study participants and their families, as well as the study investigators and their staff members who are not named as authors but who substantially contributed to the study, in particular Jean-Pierre Arsène (Pediatric Office, Le Havre, France), Jean-Paul Boyes (Medical Office, Seysses, France), Poh Chong Chan (National University Hospital, Singapore), Yu-Lung Lau (The University of Hong Kong), Christian Petit (Medical Office, Draguignan, France), Kumudhini Rajasegaran (KK Women's and Children's Hospital, Singapore) and Denis Taminau (Medical Office, Le Bourg Rosiers d'Egletons, France). In addition, the authors would like to thank the following contributors to the study and/or publication: *from the Chinese University of Hong Kong:* Edmund Anthony Severn Nelson, Paul Kay-sheung Chan, Albert Martin Li, Eva Lai-wah Fung, Assunta Chi-hang Ho, Ho-chung Yau, Kate Ching-ching Chan, Grace Pui-king Chiang, Terence Ping-yuen Ma, Alice Ka-wah Yiu, Yuen-mei Chan, Wanny Tze-wan Leung, Elsa Sau-ping Chan, Clara Yuen-fan Yu, Lucia Sui-shan Ko, Nancy Shuk-man Li, Suk-Tak Lee and Po-yin Cheng; *from the University of Hong Kong*: Tsz-leung Lee, Marco Hok-kung Ho, Brian Hon-yin Chung, Chun-yin Chong, Pamela Pui-wah Lee, Herman Hoi-ham Tsang, Winnie Wai-sim Lau, Sau-man Chan, Stella Yuk-suim Lau, Amanda Siopeng Mok, Wai-fong Wong; *from the National Healthcare Group Polyclinics, Singapore*: Simon Lee.

The authors are indebted to the global and regional clinical teams of GSK for their overall contribution to the design and conduct of the study, the running of the laboratory analyses, the coordination of the data management and statistical analyses, and the writing of the clinical protocol and the clinical study report. The authors especially thank Bruno Salaun and Sylviane Poncelet for their contribution in the study and manuscript development. The authors would also like to thank Business & Decision Life Sciences platform for writing support, editorial assistance and manuscript coordination, on behalf of GSK. Thibaud André coordinated manuscript development and editorial support. Pierre-Paul Prévot provided medical writing support.

Contributors

All authors contributed to study design, acquisition of data, statistical analyses and/or interpretation of data. All authors had full access to the complete final study reports, reviewed the manuscript draft(s), and gave final approval to submit for publication.

Funding

GlaxoSmithKline Biologicals SA funded this study and was involved in all stages of study conduct, including analysis of the data. GlaxoSmithKline Biologicals SA also took in charge all costs associated with the development and publication of this manuscript. All authors participated in the design or implementation or analysis, and interpretation of the study; and the development of this manuscript. All authors had full access to the data and gave final approval before submission.

Conflicts of interest

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf and declare the following potential conflicts of interest. Ting Fan Leung, Fong Seng Lim, Bee Wah Lee, Ngiap Chuan Tan or their respective institutions received grant for the conduct of the clinical trial from the GSK group of companies. Ting Fan Leung received honorarium as speaker from PT Wyeth Nutrition Indonesia, honorarium as

consultant from Merck Sharp & Dohme (Asia) Limited and sponsorship for attending conferences from the GSK group of companies and Nestle outside of the submitted work. Fong Seng Lim received support for travel to meetings from the GSK group of companies outside of the submitted work. Franck Thollot received personal fees during the conduct of the study from the GSK group of companies. Lars Rombo received honorarium for lectures from the GSK group of companies and from Sanofi-Pasteur MSD. Bee Wah Lee received honorarium for lectures from the GSK group of companies. Roman Rouzier received consulting fees from the GSK group of companies. Stéphanie De Simoni, Pemmaraju Suryakiran, Nicolas Folschweiller and Frank Struyf are employees of the GSK group of companies. Florence Thomas was a GSK employee at the time of the study conduct. Marjan Hezareh was a Chiltern International consultant for the GSK group of companies at the time of the study conduct. She is now an employee of Takeda Pharmaceuticals. Nicolas Folschweiller, Florence Thomas, Stéphanie De Simoni and Frank Struyf received restricted shares from the GSK group of companies. Anthony Liu and Helen May Lin Oh have no conflict of interest to declare.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2017.11.034.

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