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Long-term persistence of systemic and mucosal immune response to HPV-16/18 AS04-adjuvanted vaccine in preteen/adolescent girls and young women

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Vaccination against oncogenic human papillomavirus (HPV) types is one key intervention for cervical cancer prevention. This follow-up study assessed the persistence of the systemic and mucosal immune responses together with the safety profile of the HPV-16/18 AS04-adjuvanted vaccine administered to young women aged 10–25 years. Serum and cervicovaginal secretion (CVS) samples were collected at prespecified time-points during the 48-month follow-up period. Anti-HPV-16/18 antibody levels in serum and CVS were measured by enzyme-linked immunosorbent assay (ELISA). At Month 48, all subjects remained seropositive for serum anti-HPV-16 and -18 antibodies. As previously observed, anti-HPV-16 and -18 antibodies levels (ELISA Units/mL) were higher in subjects vaccinated at the age of 10–14 years (2862.2 and 940.8) compared to subjects vaccinated at the age of 15–25 years (1186.2 and 469.8). Moreover, anti-HPV-16 and -18 antibodies in CVS were still detectable for subjects aged 15–25 years (84.1% and 69.7%, respectively). There was a strong correlation between serum and CVS anti-HPV-16 and -18 antibodies levels (correlation coefficients = 0.84 and 0.90 at Month 48, respectively) supporting the hypothesis of transudation or exudation of serum immunoglobulin G antibodies through the cervical epithelium. The HPV-16/18 AS04-adjuvanted vaccine had a clinically acceptable safety profile. In conclusion, this follow-up study shows that the HPV-16/18 AS04-adjuvanted vaccine administered to preteen/adolescents girls and young women induces long-term systemic and mucosal immune response and has a clinically acceptable safety profile up to 4 years after the first vaccine dose.

Cervical cancer is the second most common cancer among women worldwide, with nearly 500,000 new cases and approximately 270,000 deaths each year.[1,2] Human papillomavirus (HPV) infection with oncogenic types is well recognized as the necessary cause of virtually all cervical cancers, and HPV DNA has been found in 99.7% of all cases.[2–4] HPV types 16 and 18 are the most common oncogenic HPV types, responsible for about 70% of all cervical cancers.[5,6]

Key words: cervical cancer, human papillomavirus, HPV-16/18 adjuvanted vaccine, long-term immune response, cervicovaginal secretion

Abbreviations: AE: adverse event; ATP: according-to-protocol; CI: confidence interval; CVS: cervicovaginal secretion; ELISA: enzyme-linked immunosorbent assay; EL.U: ELISA Units; GCP: good clinical practice; GMT: geometric mean titer; GSK: GlaxoSmithKline; HPV: human papillomavirus; IgG: immunoglobulin G; NOCD: new onset of chronic disease; R: correlation coefficient; SAE: serious adverse event; TVC: total vaccinated cohort; VLP: virus-like-particle

Conflict of interest: C. Pedersen has conducted vaccine trials and clinical research studies with SanofiPasteur, Wyeth, GlaxoSmithKline, Merck Sharpe & Dohme, Tibotec, Roche, ScheringPlough, Pfizer, and BristolMyersSquibb. He received financial support from GSK Biologicals through his institution to conduct our study as well as travel grants and honoraria for courses and conference. M. Lehtinen obtained grants through his employers the National Institute for Health and Welfare and University of Tampere from GSK Biologicals and Merck&Co., Inc. G. Strauss received financial support from GSK Biologicals through her institution to complete our study and declared no personal conflict of interest. T. Petäjä and A. Poder declared they have no conflict of interest. G. Catteau, F. Thomas and D. Descamps are GlaxoSmithKline Biologicals' employees. F. Thomas and D. Descamps own GSK Biologicals stock options.

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In their lifetime, up to 80% of women will acquire an HPV infection.[7,8] The majority of these natural infections resolve within two years,[8,9] but the induced antibody levels are low and many women do not seroconvert at all.[10,11] Moreover, the prevalence and the persistence of infection with oncogenic HPV types appear to be higher compared to nononcogenic HPV types.[7,9,12] Immunity conferred by natural infection may not reliably protect against (re)-infection, hence, vaccination against oncogenic HPV types is important for the overall strategy of cervical cancer prevention.

To this end, GlaxoSmithKline (GSK) Biologicals has developed a L1 protein virus-like-particle (VLP) vaccine (Cervarix®) against oncogenic types HPV-16 and HPV-18 formulated with the AS04 adjuvant system.[13] In previous trials, the HPV-16/18 AS04-adjuvanted vaccine (HPV-16/18 vaccine) was shown to have a clinically acceptable safety profile,[14] to be immunogenic[15,16] and to prevent incident and persistent HPV-16/18 infection and associated cervical neoplasia.[16–20] In addition, the HPV-16/18 vaccine has shown cross-protection against some nonvaccine oncogenic HPV types, including HPV -31, -33, and -45.[16,17,19,21] Long-term efficacy and immunogenicity, along with a clinically acceptable safety profile, were demonstrated up to 7.3 years after vaccination of women aged 15–25 years with this vaccine.[18,19,22] In women aged 15–55 years, the HPV-16/18 vaccine induced a robust immune response, in serum and cervicovaginal secretions (CVS), which has been shown to persist for at least 24 months.[23]

The mechanism by which the HPV-16/18 vaccine induces protection is not completely understood. The HPV-16/18 vaccine prevents HPV infection of basal cells in the cervical epithelium, likely through the induction of high and sustained titers of neutralizing immunoglobulin G (IgG) antibodies. The level of serum antibodies induced by HPV-vaccination is 10–100 times higher than that following natural infection.[16,18,19,24,25] Most vaccine-induced genital tract antibodies are reported to derive from the circulation by transudation or exudation across the cervical epithelium to the cervical mucus, where they bind to the HPV's outer shell (capsid) and prevent infection of host cells.[26–28] These antibodies may, however, also be actively transported or locally produced in the cervical mucosa by a mucosal immunization.[29]

Vaccination against oncogenic HPV types before sexual debut is important since 50% of HPV infections in women are acquired during the first three years of their sexually active lives[30,31] and the incidence of HPV infections remains high up to ten years after sexual debut.[7,32,33] Moreover, the highest rates of HPV infections have consistently been found in women younger than 25 years of age. The duration of vaccine-induced protection is therefore critical as women are at risk of infection with an oncogenic HPV type throughout their sexually active life.[8,34,35]

The initial phase of our study (Pedersen *et al.*,[15] Month 7) provided evidence that the HPV-16/18 vaccine elicited higher anti-HPV-16/18 antibody levels in preteen/adolescent girls (aged 10–14 years) as compared to young adult women (aged 15–25 years) and had a clinically acceptable safety profile.

Here, we present extended follow-up data from that study where the persistence of the serum and mucosal immune response and the safety profile of the HPV-16/18 vaccine have been evaluated up to 4 years after vaccination of preteen/adolescent girls and young adult women.

Material and Methods

Study objectives

The primary objective of this follow-up study was to evaluate the long-term immunogenicity of the HPV-16/18 vaccine in young women (vaccinated at the age of 10–25 years with three vaccine doses) who completed a visit 48 months after the first vaccine injection (Month 48).

The secondary objectives were: (*i*) to compare the immune responses to the HPV-16/18 vaccine in sera from subjects enrolled in our study with responses measured in sera from adults of previous studies in which efficacy has been shown;[16,19,22] (*ii*) to evaluate anti-HPV-16 and -18 antibodies responses in CVS samples and to compare the antibody levels in CVS with antibody levels in sera from subjects vaccinated pre- and postmenarche; and (*iii*) to evaluate the safety of the HPV-16/18 vaccine during long-term follow-up.

Study design

The primary study took place from September 2004 to July 2005 in 17 centers in Denmark, Estonia, Finland, Greece, The Netherlands, and Russia (Fig. 1).

The primary study included healthy female subjects divided in two age groups: the 10–14 years age group (subjects aged 10–14 years at the time of first vaccine injection) and the 15–25 years age group (subjects aged 15–25 years at the time of first vaccine injection). Participants of the 15–25 years age group were randomized (1:1:1) to receive one of three consecutive production lots of the industrial scale HPV-16/18 vaccine. A randomization blocking scheme was used to ensure that treatments were assigned equally and randomly. All participants of the 10–14 years age group received vaccine from the same production lot. Another group of women aged 15–25 years received an HPV-16/18 vaccine prepared using a modified manufacturing process and were not included in the publication.[15]

The extension study was conducted in Denmark, Estonia and Finland from June 2006 to January 2009 as a phase III, open, multicentric, follow-up study (NCT00337818) designed to evaluate the safety and immunogenicity of the HPV-16/18 vaccine up to Month 48 in subjects vaccinated at the age of 10–25 years (Fig. 1).

Study population and ethics

Participants were enrolled in the primary study if: (*i*) they were abstinent from sexual activity, or were using adequate contraceptive precautions for 30 days before vaccination and

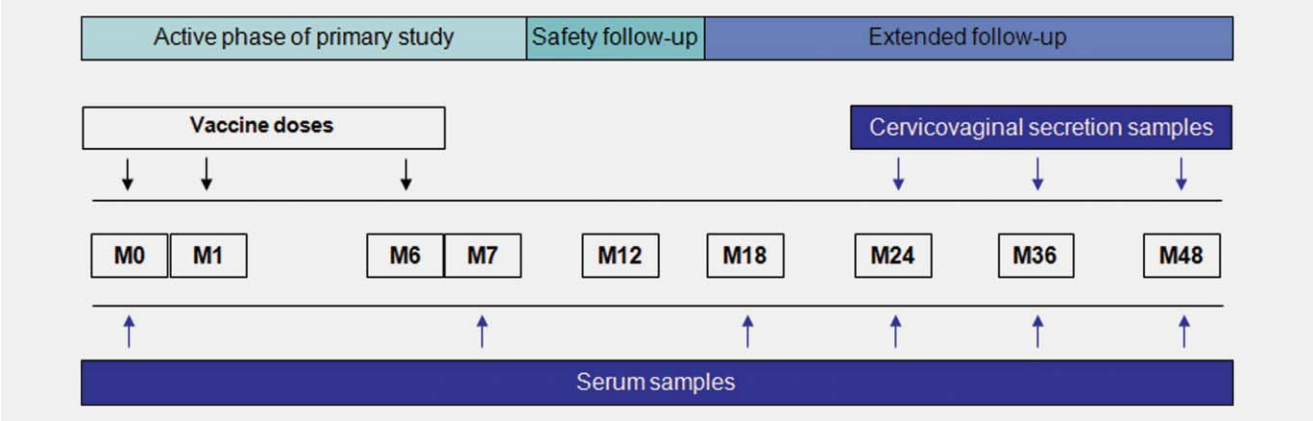

Figure 1. Primary and extended follow-up study design: Vaccine administration, serologic and cervicovaginal secretion evaluation. M: Month.

up to 2 months after completion of the vaccination series; (*ii*) they had negative pregnancy test results (if they were of childbearing potential); and (*iii*) they had no more than six lifetime sexual partners. Individuals were excluded from the extension study at the time of study entry if they had used an investigational product, a nonregistered product or chronic immune-modifying drugs. Exclusion criteria included also the administration of immunoglobulins or blood products within 3 months prior to a blood sampling.

To be eligible for the extension study, the subjects had to have participated in the primary study in Denmark, Estonia or Finland, to have received three doses of the HPV-16/18 vaccine (at Months 0, 1 and 6) and to have completed the Month 7 visit.[15] Subjects who missed assessment at Month 18 (*i.e.*, first visit of the extension study) were eligible to join the study at Month 24.

All participants had to sign the written informed consent before enrollment. For subjects below the legal age of consent, written informed consent had to be obtained from a parent or legally acceptable representative and, in addition, the subject had to sign and personally date a written informed assent.

The study protocol, any amendments, the informed consent and other information that required preapproval were reviewed and approved by a national, regional, or investigational centre Ethics Review Committee or Institutional Review Board.

Our study was conducted in accordance with good clinical practice (GCP) and all applicable regulatory requirements, including, where applicable, the Declaration of Helsinki. All distributed material had received prior approval by the Ethics Review Committees.

Study vaccines

The HPV-16/18 vaccine (GSK Biologicals, Rixensart, Belgium) contains HPV-16 and -18 L1 proteins self-assembled as VLP and is formulated with AS04, an adjuvant system known to enhance the vaccine's immunogenicity.[36] Each dose of the HPV-16/18 vaccine contains 20 μg of each HPV-16 and -18 L1 proteins adjuvanted with 550 μg of AS04 (500 μg aluminium hydroxide and 50 μg 3-O-desacyl-4′-monophosphoryl lipid A). The HPV-16/18 vaccine was supplied in individual 0.5-mL prefilled syringes and administered into the deltoid muscle on a 0-, 1- and 6-month schedule (Fig. 1).[15]

Serologic and CVS evaluations

In the follow-up study, blood samples were collected at Months 18, 24, 36 and 48 for measurement of anti-HPV-16 and -18 antibody titers in serum (Fig. 1). All blood samples were evaluated for anti-HPV-16 and -18 antibodies using type-specific enzyme-linked immunosorbent assay (ELISA) at GSK Biologicals Laboratories, Rixensart, Belgium.

Anti-HPV-16 and -18 antibodies were also measured in CVS samples collected at Months 24, 36 and 48 in postmenarcheal subjects who volunteered for the procedure (Fig. 1). CVS samples were collected using ophthalmic sponges (Merocel® Eye Spear or Sponge Points [Medtronic Inc; Jacksonville, Florida, USA]). The sponge was placed in contact with the cervix for 30–60 seconds to absorb mucus. Antibody extraction from CVS samples was performed at GSK Biologicals Laboratories, Rixensart, Belgium and anti-HPV-16 and -18 IgG antibodies were detected and quantified according to ELISA serum standardized protocols as previously published.[16,23,37]

To avoid any bias in results, the presence of blood in CVS samples was evaluated using the Hemastix® (Bayer Healthcare LLC) reagent strip test. After extraction of antibodies from CVS by two washing steps, a fixed volume of extracted sample was dispensed onto the strip test end. After one minute, the color of the test pad was matched to the color chart on the bottle label. Results were expressed as 0, 10, 25, 80, or 200 erythrocytes per μL.

Vaccine safety

The occurrence of serious adverse events (SAEs), medically significant adverse events (AEs), new onset of chronic

diseases (NOCDs) and pregnancies was recorded throughout the entire study period. AEs, withdrawal due to AE(s), pregnancies and their outcomes were described in detail. SAEs were further evaluated for their clinical relevance and relationship to vaccination.

SAEs were defined as any untoward medical occurrence that was life-threatening, required hospitalization, resulted in disability or incapacity, was an important medical event, resulted in death, or was a congenital anomaly/birth defect in the offspring of a study participant. Medically significant AEs were defined as adverse events prompting emergency room or physician visits, which were not related to common diseases or routine visits for physical examination or vaccination or SAEs not related to common diseases. NOCDs included autoimmune conditions, allergies and asthma.

Statistical methods

The primary immunogenicity analysis was performed on the according-to-protocol (ATP) immunogenicity cohort which included all evaluable subjects, *i.e.*, subjects who were included in the primary study ATP immunogenicity analyses, meeting all eligibility criteria, complying with the procedures and intervals defined in the protocol with no elimination criteria during the study and for whom serology results were available for a particular blood sampling time point (at Month 18, 24, 36 or 48) of the extension phase. A supplementary analysis was performed on the Total Vaccinated cohort (TVC) which included all vaccinated subjects who received three doses of HPV-16/18 vaccine in the primary study and for whom data were available.

Seropositivity rates (with 95% confidence interval [CI]) were calculated for anti-HPV-16 and -18 in both groups. Seropositivity was defined as a titer greater than or equal to the assay threshold established at 8 ELISA Units/mL (EL.U/mL) for anti-HPV-16 and 7 EL.U/mL for anti-HPV-18.[15,19] The range and distribution of antibody concentrations in serum were tabulated by geometric mean titers (GMTs) and their 95% CI for anti-HPV-16 and -18 at each time-point.

In the absence of an accepted serological correlate of protection, descriptive comparisons were performed between anti-HPV-16 and -18 GMTs in our study and anti-HPV-16 and -18 GMTs in women aged 15–25 years who cleared a natural infection and mounted an immune response in the Phase III PATRICIA trial.[16] The immunogenicity results were also compared to the anti-HPV-16 and -18 GMTs from the plateau phase of the HPV-007 efficacy study at Months 45–50.[19,22]

For the subjects who volunteered to provide CVS samples, correlation between serum and CVS antibody concentrations was calculated by Pearson coefficient. To minimize the antibody titer variation during the menstrual cycle, antibody titers (expressed in EL.U/mL) measured in CVS and in serum were divided by the amount of total IgG measured in μg/mL.[28] This ratio (expressed in EL.U/μg) was used for the correlation. CVS samples with Hemastix® value >200 erythrocytes per μL were excluded from analyses.

Safety analyses were performed on the TVC. The percentages of subjects reporting at least one SAE, NOCD or other medically significant AE throughout the study (between Month 0 and Month 48) were tabulated with their exact 95% CI per treatment group.

The analyses were performed using Statistical Analysis System 9.1 and Proc StatXact 7.0.

Results

Study population

Of the 616 subjects who received at least one dose of HPV-16/18 vaccine prepared using standard manufacturing process in the primary study, 321 were from the three countries that accepted to participate in the extension study. Of these, 243 subjects entered the extension study, 220 subjects (51 aged 10–14 years and 169 aged 15–25 years at the first vaccination) attended the visit at Month 48 and 193 subjects (50 from the 10–14 years age group and 143 from the 15–25 years age group) were included in the ATP cohort for immunogenicity (Fig. 2).

At Month 48, the mean ages of participants (TVC cohort) were 15.7 years for the group of subjects aged 10–14 years at the time of first vaccine injection and 24.2 years for the group of subjects aged 15–25 years at the same time. Almost all subjects were White/Caucasian (98.2%). When compared to the demographic characteristics of the primary study, subjects in the two age groups participating in the extension study were of similar age and heritage (data not shown).

Immunogenicity

Most subjects of the 10–14 and 15–25 years age groups were seronegative for anti-HPV-16 antibodies (100% and 87.2%, respectively) and for anti-HPV-18 antibodies (95.8% and 87.2%, respectively) prior to vaccination. All initially seronegative subjects had seroconverted after vaccination and remained seropositive for both antibodies up to 4 years after the first vaccine dose (Table 1).

Immunological kinetic profiles showed that anti-HPV-16/18 antibodies peaked at Month 7, then gradually declined in all age groups tending towards a plateau (Fig. 3). At Month 48, GMTs (EL.U/mL) (95% CI) for anti-HPV-16 in initially seronegative subjects were 2862.2 (2129.3–3847.3) in the 10–14 years age group and 1186.2 (1007.4–1396.8) in the 15–25 years age group (Fig. 3*a*). Anti-HPV-18 antibody GMTs were respectively 940.8 (714.8–1238.3) and 469.8 (394.7–559.2) for the same age groups and time-point (Fig. 3*b*). At all time-points postvaccination, GMTs were higher in subjects of the 10–14 years age group compared to subjects of the 15–25 years age group for anti-HPV-16 antibodies (between 2.4- and 2.9-fold) and anti-HPV-18 antibodies (between 2.0- and 2.5-fold).

In initially seronegative subjects of the 10–14 and 15–25 years age groups, anti-HPV-16 antibody GMTs at Month 48

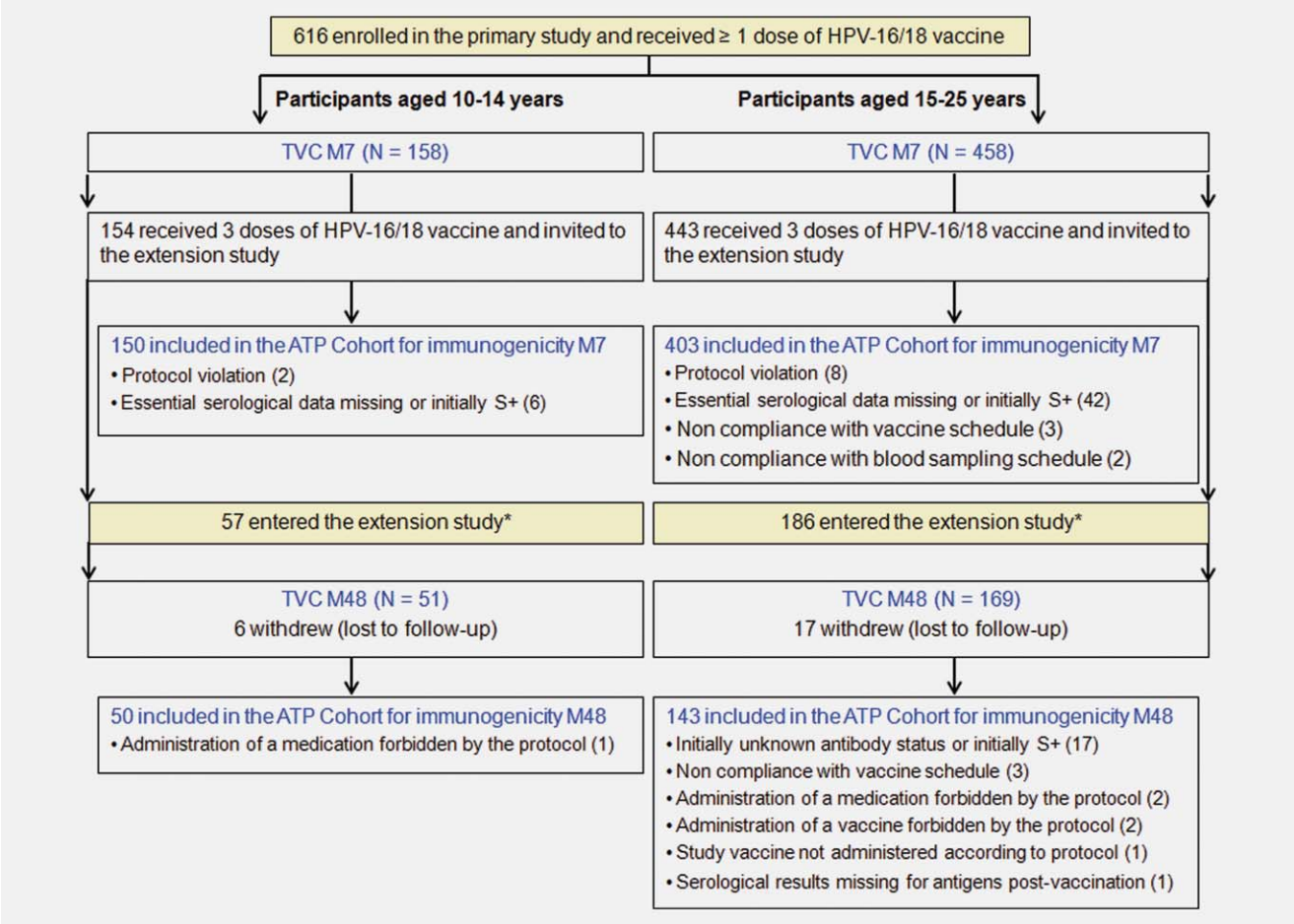

Figure 2. Flow of participants through the primary and extension study and definition of analyses cohorts. *Greece, Russia and The Netherlands declined participation in the extension study. TVC: total vaccinated cohort; ATP: according-to-protocol; N: number of subjects; M7: Month 7; M48: Month 48; S+: seropositive.

were, respectively, 96.0- and 39.8-fold higher than anti-HPV-16 antibody levels achieved in subjects who cleared a natural infection and mounted an immune response in the Phase III PATRICIA trial (Fig. 3*a*).[16] Anti-HPV-18 antibody GMTs were respectively 41.4- and 20.7-fold higher than levels after a natural infection for the same age groups (Fig. 3*b*). Compared to the plateau level observed in subjects from another study in which sustained efficacy of the HPV-16/18 vaccine has been demonstrated,[19,22] anti-HPV-16 antibody titers were respectively 7.2- and 3.0-fold higher in the 10–14 years and the 15–25 years age groups (Fig. 3*a*). Anti-HPV-18 antibody titers were ~3.2- and 1.6-fold higher than the plateau level for the same age groups (Fig. 3*b*).

At Month 48, anti-HPV-16 and -18 antibody testing in CVS was performed on 69 and 66 samples (with Hemastix® $<$ 200 erythrocytes/μL) from subjects of the 15–25 years age group. Anti-HPV-16 and -18 antibodies were detected in CVS from 84.1% (95% CI: 73.3, 91.8) and 69.7% (95% CI: 57.1, 80.4) of subjects. Similar data were observed in CVS samples tested at Month 24 and Month 36.

Serum anti-HPV-16 and -18 IgG antibody response was evaluated at different time-points in subjects with and without detectable anti-HPV-16/18 IgG antibodies in their CVS at Month 48 (Table 2). All subjects remained seropositive for anti-HPV-16 and -18 antibodies at Month 48. However, subjects with detectable cervicovaginal anti-HPV-16 IgG antibodies displayed higher anti-HPV-16 serum GMTs than subjects without detectable antibodies in CVS. The difference in anti-HPV-16 GMTs between subjects with and without detectable cervicovaginal antibodies increased with time (from 1.7 fold at Month 7 to 3.2 fold at Month 48). A comparable trend was observed for anti-HPV-18 IgG antibodies.

A strong and direct correlation was observed between antibody levels in serum and CVS from the subjects of the 15–25 years age group throughout the study. The correlation coefficients (R) between antibody levels in serum and CVS for anti-HPV-16 antibodies were 0.93, 0.91 and 0.84 at Month 24, 36 and 48, respectively. For anti-HPV-18 antibodies the correlation coefficients were 0.93, 0.91 and 0.90 for the same time-points (Fig. 4).

Table 1. Seropositivity rates and GMTs for serum HPV-16/18 IgG antibodies by pre-vaccination status (ATP immunogenicity cohort Month 48)

Antigen	S	Month	10–14 years age group			15–25 years age group		
			N	Seropositivity rate (95% CI)	GMT (EL.U/mL) (95% CI)	N	Seropositivity rate (95% CI)	GMT (EL.U/mL) (95% CI)
HPV-16	S−	PRE	49	0.0 (0.0, 7.3)	4.0 (4.0, 4.0)	123	0.0 (0.0, 3.0)	4.0 (4.0, 4.0)
		M7	49	100 (92.7, 100)	21271.1 (16,604.8, 27,248.8)	123	100 (97.0, 100)	7376.4 (6,288.9, 8.651.9)
		M24	49	100 (92.7, 100)	4074.0 (3,026.9, 5,483.5)	117	100 (96.9, 100)	1426.2 (1,206.7, 1,685.6)
		M36	48	100 (92.6, 100)	3444.9 (2580.5, 4598.7)	118	100 (96.9, 100)	1372.1 (1,162.8, 1,619.0)
		M48	49	100 (92.7, 100)	2862.2 (2129.3, 3847.3)	123	100 (97.0, 100)	1186.2 (1007.4, 1396.8)
	S+	PRE	–	–	–	18	100 (81.5, 100)	29.1 (17.1, 49.6)
		M7	–	–	–	18	100 (81.5, 100)	3250.1 (1,947.1, 5,425.1)
		M24	–	–	–	17	100 (80.5, 100)	1075.7 (643.4, 1,798.6)
		M36	–	–	–	17	100 (80.5, 100)	1149.4 (664.3, 1,988.9)
		M48	–	–	–	18	100 (81.5, 100)	874.7 (501.5, 1,525.4)
HPV-18	S−	PRE	46	0.0 (0.0, 7.7)	3.5 (3.5, 3.5)	123	0.0 (0.0, 3.0)	3.5 (3.5, 3.5)
		M7	46	100 (92.3, 100)	8179.0 (6,322.3, 10,580.9)	123	100 (97.0, 100)	3263.3 (2,789.4, 3817.7)
		M24	46	100 (92.3, 100)	1412.3 (1,078.5, 1,849.5)	117	100 (96.9, 100)	616.4 (515.1, 737.5)
		M36	45	100 (92.1, 100)	1188.3 (887.4, 1,591.4)	118	100 (96.9, 100)	575.2 (480.5, 688.7)
		M48	46	100 (92.3, 100)	940.8 (714.8, 1,238.3)	123	100 (97.0, 100)	469.8 (394.7, 559.2)
	S+	PRE	2	100 (15.8, 100)	8.5 (4.0, 17.9)	18	100 (81.5, 100)	34.4 (18.2, 65.0)
		M7	2	100 (15.8, 100)	5174.6 (4.3, 6,201,326)	18	100 (81.5, 100)	2167.5 (1,406.4, 3,340.7)
		M24	2	100 (15.8, 100)	1360.5 (1.4, 1,337,388)	17	100 (80.5, 100)	639.4 (340.2, 1,201.5)
		M36	2	100 (15.8, 100)	859.7 (5.0, 149,083.1)	18	100 (81.5, 100)	495.6 (281.0, 874.1)
		M48	2	100 (15.8, 100)	824.7 (0.9, 758,371.6)	18	100 (81.5, 100)	414.6 (231.4, 742.8)

Abbreviations: GMT, geometric mean antibody titer calculated on all subjects; N, number of subjects with pre-vaccination results available; PRE, Pre-vaccination; M7, Month 7; M24, Month 24; M36, Month 36; M48, Month 48; 95% CI, 95% confidence interval, S, pre-vaccination status; S−/S+, seronegative/seropositive subjects (antibody titre $</\geq$ 8 and 7 EL.U/mL for anti-HPV-16 and -18 IgG antibodies, respectively) prior to vaccination.

Safety

Throughout the study (Month 0 to Month 48), a total of 27 SAEs were reported by 23 subjects. Four subjects (2.5% CI: 0.7, 6.4) reported 4 SAEs in the 10–14 years age group and 19 subjects (4.1% CI: 2.5, 6.4) reported 23 SAEs in the 15–25 years age group. None of these events was considered to be possibly related to the study vaccination by the investigator.

Twenty-two subjects (13.9% CI: 8.9, 20.3) reported 24 medically significant AEs in the 10–14 years age group and 92 subjects (20.1% CI: 16.5, 24.1) reported 144 medically significant AEs in the 15–25 years age group. The most frequently reported medically significant AEs were depression (11), cystitis (9), asthma (6) and acne (5).

Three subjects (1.9% CI: 0.4, 5.4) in the 10–14 years age group and 19 subjects (4.1% CI: 2.5, 6.4) in the 15–25 years age group reported respectively 3 and 20 NOCDs (based on GSK assessment). The most frequently reported NOCDs were asthma (5) and hypothyroidism (3). None of these events were considered as possibly related to study vaccination by the investigator.

Of 45 pregnancies reported (one in the 10–14 years age group and 44 in the 15–25 years age group), there were 35 normal births, one stillbirth, one therapeutic abortion and one missed abortion in the same subject, four elective terminations and three premature infants.

Discussion

This long-term follow-up study was designed to evaluate the immunogenicity and safety of the HPV-16/18 vaccine in healthy female subjects (aged 10 to 25 years at the first vaccine injection) up to four years after administration of the first vaccine dose. The vaccine had a clinically acceptable safety profile and high serum and mucosal anti-HPV-16 and -18 antibody levels were observed up to Month 48.

All initially seronegative subjects seroconverted and remained seropositive for both anti-HPV-16 and -18 antibodies throughout the study, regardless of their age at vaccination. The immunological kinetic profiles, showing peak titers gradually declining and tending towards a plateau in all age groups, were similar to those observed in other HPV-16/18 vaccine clinical efficacy trials.[16,19,23] In line with previous studies, anti-HPV-16 and -18 serum antibody titers at Month 48 were still substantially higher than titers elicited by a

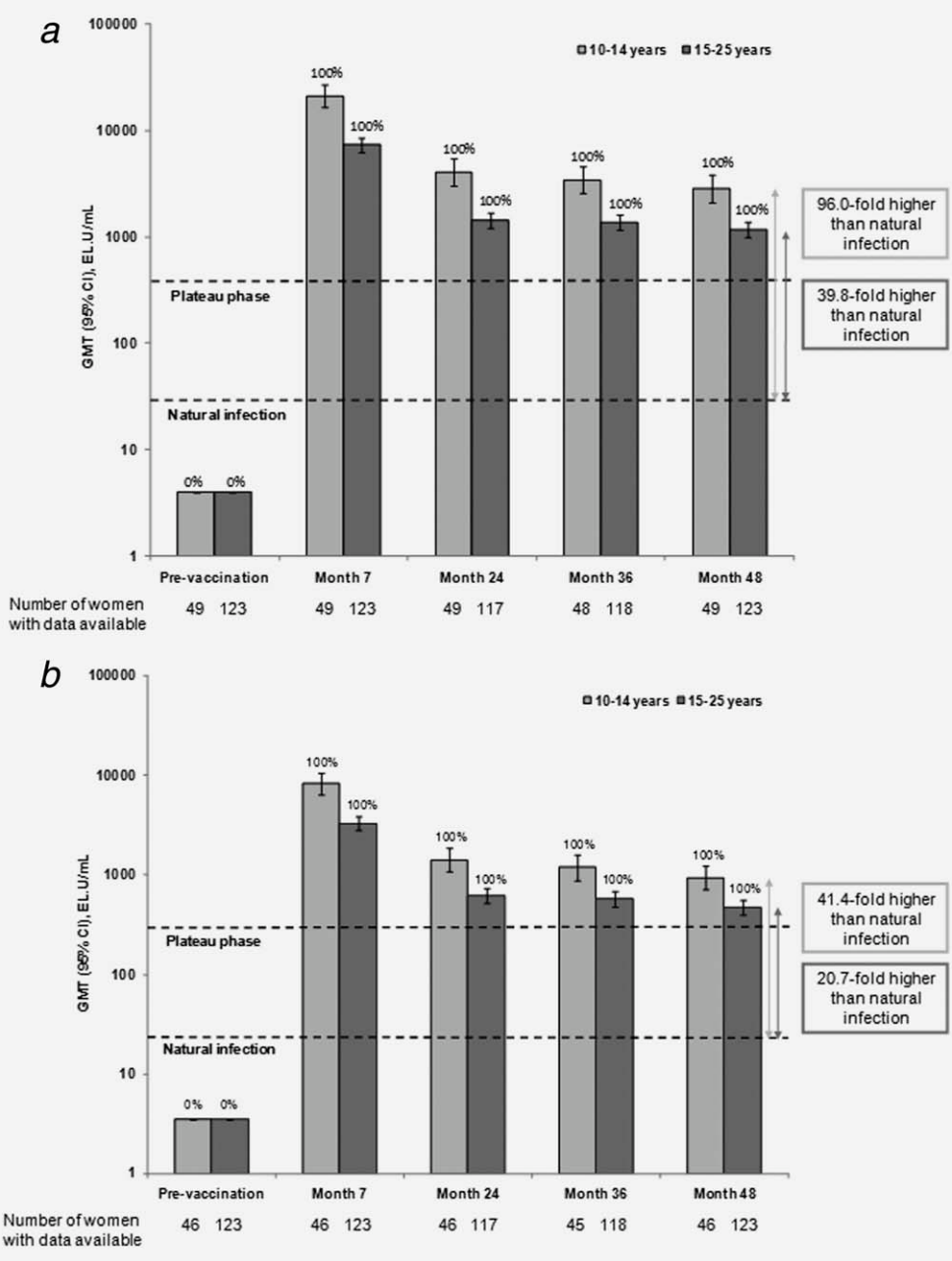

Figure 3. Geometric mean titers of anti-HPV-16 (*a*) and -18 (*b*) antibodies in initially seronegative subjects aged 10–14 years and 15–25 years at the time of first vaccination (ATP immunogenicity cohort Month 48). 10–14 years: subjects aged 10–14 years at the time of first vaccine dose; 15–25 years: subjects aged 15–25 years at the time of first vaccine dose; GMT: geometric mean titer; Error bars: 95% CI: 95% confidence interval; Seropositivity rates shown above bars. Results of Month 18 were not presented since only eight subjects attended the visit. Natural infection: GMTs of subjects from Study HPV-008 who were HPV-16 (*a*) or -18 (*b*) DNA negative and seropositive at baseline (*i.e.*, who had cleared a natural infection; GMT: 29.8 EL.U/mL (*a*) or GMT: 22.7 EL.U/mL (*b*))[16]Plateau phase: GMTs of subjects from Study HPV-007 in women aged 15–25 years at Months 45–50 after the first vaccine dose (Total cohort; GMT: 397.8 EL.U/mL (*a*) or GMT: 297.3 EL.U/mL (*b*))[19,22]

natural infection,[16] and they were above antibody levels associated with sustained protection observed in previous clinical trials.[19,22] This high and sustained immune response induced by the vaccine may in part be explained by the presence of the AS04 adjuvant system in the vaccine formulation.[36]

Results from the primary study showed that the vaccine induced higher antibody titers when administered to young adolescents aged 10–14 years as compared to young women aged 15–25 years.[15] The results of Month 7 were not unexpected since immune response to vaccination is known to

Table 2. Comparison of serum antibody response in HPV-16/18 vaccinated young women with and without detectable cervicovaginal anti-HPV-16/18 IgG antibodies at Month 48 (TVC, 15–25 years age group)

Anti-HPV-16/18 IgG	Month	HPV-16			HPV-18		
		N	Seropositivity rate (95% CI)	GMT (EL.U/mL) (95% CI)	*N*	Seropositivity rate (95% CI)	GMT (EL.U/mL) (95% CI)
<LOQ	PRE	11	27.3 (6.0, 61.0)	5.4 (3.8, 7.7)	19	31.6 (12.6, 56.6)	7.5 (4.0, 14.1)
	M7	11	100 (71.5, 100)	4173.5 (2782.2, 6260.7)	20	100 (83.2, 100)	1648.3 (1204.8, 2255.1)
	M24	11	100 (71.5, 100)	652.6 (381.7, 1115.7)	18	100 (81.5, 100)	228.4 (174.4, 299.0)
	M36	9	100 (66.4, 100)	547.1 (308.1, 971.6)	18	100 (81.5, 100)	290.2 (200.7, 419.5)
	M48	11	100 (71.5, 100)	393.7 (245.3, 631.7)	20	100 (83.2, 100)	211.6 (147.7, 303.3)
≥LOQ	PRE	58	17.2 (8.6, 29.4)	6.3 (4.7, 8.5)	45	17.8 (8.0, 32.1)	6.5 (4.1, 10.2)
	M7	58	100 (93.8, 100)	7148.6 (5560.9, 9189.6)	46	100 (92.3, 100)	4011.4 (3180.5, 5059.4)
	M24	54	100 (93.4, 100)	1479.4 (1212.7, 1804.7)	44	100 (92.0, 100)	843.3 (641.5, 1108.6)
	M36	55	100 (93.5, 100)	1400.2 (1143.0, 1715.2)	43	100 (91.8, 100)	816.2 (632.3, 1053.7)
	M48	58	100 (93.8, 100)	1260.8 (1037.2, 1532.7)	46	100 (92.3, 100)	682.0 (533.2, 872.4)

Abbreviations: GMT, geometric mean titer; LOQ, limit of quantification, percent of individuals below (<) or above (≥) (8 and 7 EL.U/mL for anti-HPV-16 and -18 IgG antibodies, respectively); N, number of subjects with pre-vaccination results available; PRE, Pre-vaccination; M7, Month 7; M24, Month 24; M36, Month 36; M48, Month 48; 95% CI, 95% confidence interval.

be enhanced in younger populations compared to advancing age groups.[38–40] Of importance, the same trend was observed throughout the four years of follow-up in the extension study, which therefore brings new findings of major significance with regards to the expected long-lasting protection.

Preclinical studies have demonstrated the role of species-specific antibodies, directed against HPV L1 capsid protein, in protection against HPV infection and subsequent lesion development[41] in three distinct animal model systems (canine oral papillomavirus,[42] cottontail rabbit papillomavirus[43–45] and bovine papillomavirus[46]). One possible mechanism of protection at the mucosal surface is the transudation/exudation of serum IgG antibodies into CVS. In humans, high levels of mucosal antibodies could prevent virus particles from infecting the cervical basal cell layer at the transformation zone, which is the metaplastic zone between the squamous and columnar epithelium in the cervix where cervical cancers usually develop.[47] Serum IgG antibodies are thought to transudate or exudate at this site across the cervical epithelium.[27,48] In a previous study, systemic immunization with HPV-16 VLPs has been shown to elicit high titers of anti-HPV-16-specific IgG antibodies in cervical secretions, which were believed to be derived from serum transudation.[28] Similarly, the presence of anti-HPV antibodies at the cervix in women following administration of the HPV-16/18 vaccine has also been reported.[23,26,49] Moreover, a previous study showed that CVS antibody positivity rates for both anti-HPV-16 and -18 were higher after administration of the HPV-16/18 vaccine than after administration of a quadrivalent VLP HPV-6/11/16/18 vaccine.[50]

In our study, mucosal immune response to the HPV-16/18 vaccine was evaluated in CVS samples provided by subjects on a voluntary basis. The correlation between CVS and serum titration for antibodies against HPV-16 and HPV-18 was assessed and analysed. At Month 48, 84.1% and 69.7% of subjects vaccinated at the age of 15 to 25 years still had detectable antibody titers in their CVS samples for respectively anti-HPV-16 and anti-HPV-18. The strong correlation between serum and CVS antibodies persisted until Month 48, suggesting that transudation/exudation of serum IgG antibodies to the cervical epithelium is long-standing. Results of three other studies confirm that women who had detectable anti-HPV-16/18 antibodies in CVS reported consistently higher antibody levels in serum, regardless of age.[23,49,50]

The immune response was further evaluated regarding the presence or absence of detectable cervicovaginal anti-HPV-16 and -18 IgG antibodies 48 months after the first vaccine injection. Throughout the study, subjects with detectable CVS antibodies had higher serum GMTs for anti-HPV-16/18 IgG antibodies than subjects with no detectable cervicovaginal anti-HPV-16/18 antibodies. This suggests that the antibody transudation from serum to CVS depends on the antibody level in the serum. Indeed, even if serum antibody levels decreased with time in both groups, the drop-off was slightly higher in subjects without detectable CVS antibodies at Month 48. On the other hand, in the group without detectable CVS antibodies, lower GMTs were observed from the beginning for anti-HPV-18 and from Month 24 onwards for anti-HPV-16. In the absence of an accepted serological correlate of protection, it is unclear whether this group may respond differently to the vaccine and might turn susceptible to infections caused by different HPV types at different times in the future.

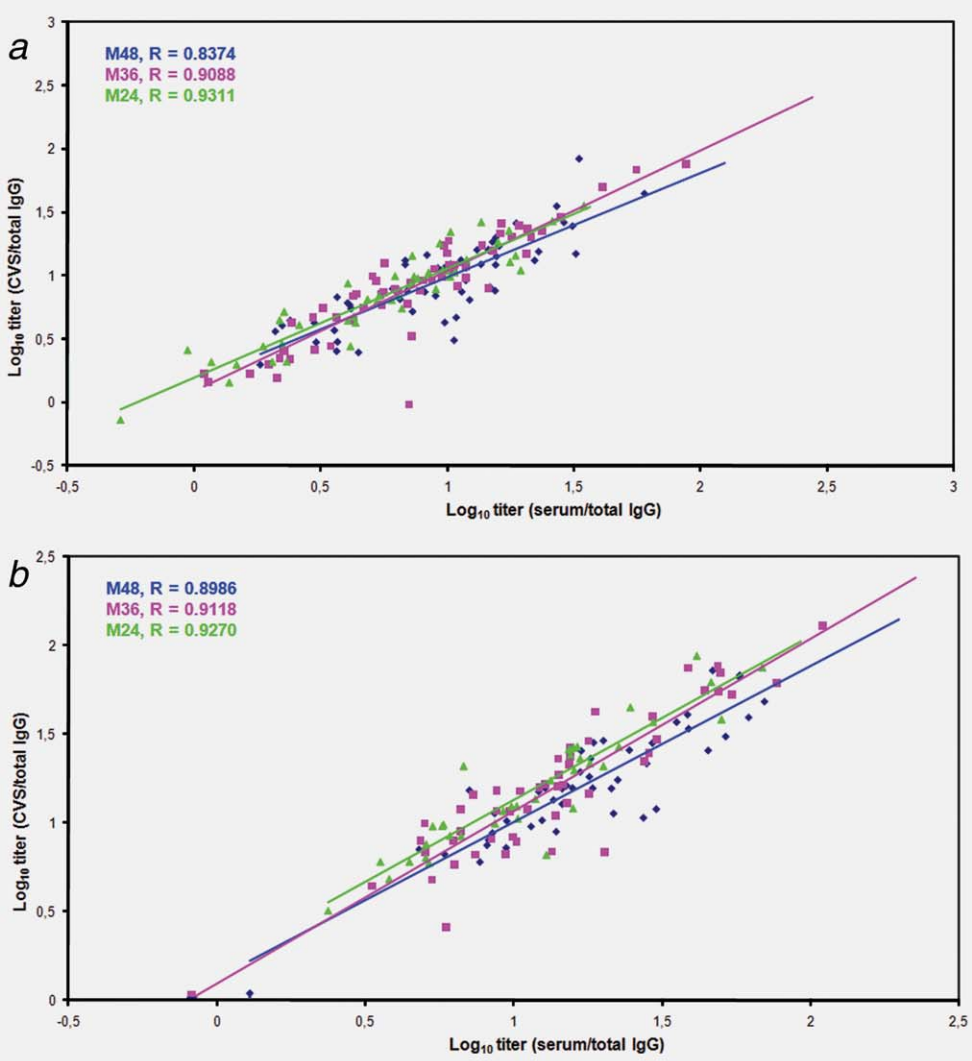

Figure 4. Correlation between antibody levels in serum and CVS samples at Month 24, Month 36 and Month 48 for anti-HPV-16 (*a*) and -18 (*b*) antibodies. The scatter plots show the ratio (specific IgG/total IgG) transformed to linear $\log_{10}$ values (TVC, 15–25 years age group). M24: Month 24; M36: Month 36; M48: Month 48; *R*: correlation coefficient.

The safety analysis revealed a clinically acceptable profile. The frequency of SAEs, medically significant AEs and NOCDs were comparable to those reported in previous analyses.[15]

The main limitations of our study were the number of subjects in the 48-month follow-up study which was lower compared to the primary study, the limited number of subjects from whom CVS samples were assessable and the fact that CVS samples were not collected since the beginning of the study. The proportion and the demographic characteristics of subjects in the initially 10–14 years and 15–25 years age groups were, however, conserved in the follow-up study.

In conclusion, the long-term immunogenicity in serum and CVS of the HPV-16/18 vaccine for both HPV-16 and HPV-18 was demonstrated. The vaccine had a clinically acceptable safety profile when administered to healthy female subjects aged 10 to 25 years. Moreover, the previously observed higher anti-HPV-16/18 antibody levels in early adolescents as compared to young adults persisted up to 4 years after the first vaccine dose. The strong correlation between levels of anti-HPV-16/18 antibodies in serum and CVS up to Month 48 supports long-term transudation or exudation of serum IgG antibodies to the cervical epithelium. These data support the administration of HPV-16/18 vaccine in preteen/adolescent girls and in young women.

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