Persistence of immune responses to the HPV-16/18 AS04-adjuvanted vaccine in women aged 15–55 years and first-time modelling of antibody responses in mature women: results from an open-label 6–year follow-up study

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Objective Evaluation of the long-term HPV-16/18 AS04-adjuvanted vaccine immunogenicity persistence in women.

Design Multicentre, open-label, long-term follow-up (NCT00947115) of a primary phase–III study (NCT00196937).

Setting Six centres in Germany and Poland.

Population 488 healthy women (aged 15–55 years, age-stratified into groups: 15–25, 26–45, and 46–55 years) who received three vaccine doses in the primary study.

Methods Immune responses were evaluated in serum and cervicovaginal secretion (CVS) samples 6 years after dose 1. Anti-HPV-16/18 geometric mean titres (GMTs) were measured by enzyme-linked immunosorbent assay (ELISA), and were used to fit the modified power-law and piecewise models, predicting long-term immunogenicity. Serious adverse events (SAEs) were recorded.

Main outcome measures Anti-HPV-16/18 seropositivity rates and GMTs 6 years after dose 1.

Results At 6 years after dose 1, all women were seropositive for anti-HPV–16 and ≥97% were seropositive for anti-HPV–18

antibodies. GMTs ranged from 277.7 to 1344.6 EU/ml, and from 97.6 to 438.2 EU/ml, for anti-HPV–16 and anti-HPV–18, respectively. In all age groups, GMTs were higher (anti-HPV–16, 9.3–45.1-fold; anti-HPV–18, 4.3–19.4-fold) than levels associated with natural infection (29.8 EU/ml). A strong correlation between serum and CVS anti-HPV-16/18 levels was observed, with correlation coefficients of 0.81–0.96 (anti-HPV–16) and 0.69–0.84 (anti-HPV–18). Exploratory modelling based on the 6–year data predicted vaccine-induced anti-HPV-16/18 levels above natural infection levels for at least 20 years, except for anti-HPV–18 in the older age group (piecewise model). One vaccine-related and two fatal SAEs were reported.

Conclusions At 6 years after vaccination, immune responses induced by the HPV-16/18 AS04-adjuvanted vaccine were sustained in all age groups.

Keywords HPV-16/18 AS04-adjuvanted vaccine, human papillomavirus, persistence.

Linked article This article is commented on by IM Linhares and SS Witkin, p. 118 in this issue. To view this mini commentary visit http://dx.doi.org/10.1111/1471-0528.13069.

Introduction

Persistent infection with oncogenic human papillomavirus (HPV) types is a necessary cause of cervical cancer, the third most common cancer in women worldwide and the fourth leading cause of cancer-related deaths in women.[1–7] In Europe, cervical cancer is the second most frequent cancer among women aged 15–44 years.[8]

Approximately 10% of women worldwide with a normal cervical cytology carry a detectable cervical HPV infection.[9,10] Although most HPV infections are transient, persistent infections may progress to pre-cancerous cervical intraepithelial neoplasia (CIN) and cervical cancer.[11–14]

Among approximately 15 high-risk (HR) oncogenic HPV types identified, HPV–16 and HPV–18 cause approximately 70% of invasive cervical cancer cases.[1,3,12,15–18] About 100 million women worldwide carry HPV-16/18 DNA, and the detection of HPV-16/18 is associated with a five-fold greater risk of developing CIN than the detection of other HR HPV types.[16,19]

Cervical HPV infections are particularly common in young, sexually active women, but women remain at risk throughout their sexually active life, with another peak observed in women aged >45 years.[10,12,20–25] Therefore, in addition to the current vaccination programmes targeting adolescent girls before their sexual debut, vaccination of women aged >25 years should be considered on an individual basis.[26]

The prophylactic HPV-16/18 AS04-adjuvanted vaccine (Cervarix®; GlaxoSmithKline, Rixensart, Belgium), which is licensed in over 100 countries worldwide,[27–30] has been shown to be immunogenic and efficacious against HPV-16/18 infections in women aged 15–25 years.[28,31–37] Recently, a high vaccine efficacy has also been demonstrated in women aged ≥26 years.[38] The vaccine was also shown to have a clinically acceptable safety profile.[27,39] In Europe, it is indicated for use in girls from 9 years of age.[40]

In a previously published primary open-label phase–III study, the HPV-16/18 AS04-adjuvanted vaccine was immunogenic and well tolerated in women aged 15–55 years; in addition, a high correlation between anti-HPV-16/18 antibody levels in sera and cervicovaginal secretion (CVS) samples was observed, regardless of age.[41] In the first follow-up of the primary study (NCT00196937), immune responses to the vaccine were sustained up to 2 years after vaccination; CVS anti-HPV-16/18 antibodies were not assessed.[30] The current extended follow-up evaluates the long-term persistence of antibody responses, up to 10 years after the first dose, in women vaccinated in the primary study. Here, we report persistence of the serum and CVS anti-HPV-16/18 antibodies, and vaccine safety, up to 6 years after vaccination. In addition, we present for the first time modelling data predicting long-term anti-HPV-16/18 antibody persistence in vaccinated women aged >25 years.

Methods

Study design

This study is a multicentre, open-label, age-stratified, long-term follow-up (LTFU) of the primary study (NCT00196937), conducted in six centres in Poland and Germany between October 2004 and July 2005, in which healthy women aged 15–55 years, stratified in three age groups (15–25, 26–45, and 46–55 years), received three doses of the HPV-16/18 AS04-adjuvanted vaccine at 0, 1, and 6 months. The immunogenicity persistence and vaccine safety will be evaluated up to 10 years after the first dose. Here, we present the results obtained up to 6 years after the first dose. The overall study design is summarised in Figure 1.

All women participating in the study provided written informed consent prior to enrolment. This study was conducted in accordance with the Good Clinical Practice guidelines and all applicable regulatory requirements, including the Declaration of Helsinki. This study has been registered at www.clinicaltrials.gov (NCT00947115).

Study objectives

The primary study objective was to evaluate the long-term immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine in terms of anti-HPV-16 and anti-HPV-18 geometric mean antibody titres (GMTs) and seroconversion rates in serum, measured by enzyme-linked immunosorbent assay (ELISA) 6 years after the first dose.

The secondary objectives included: an evaluation of immune responses to the vaccine in serum compared with antibody levels achieved after natural infection,[35,36] and compared with antibody levels achieved in other efficacy studies;[28,31–33] a comparison of anti-HPV-16/18 levels in CVS and serum samples, and an evaluation of the safety of the vaccine up to 6 years following the first dose.
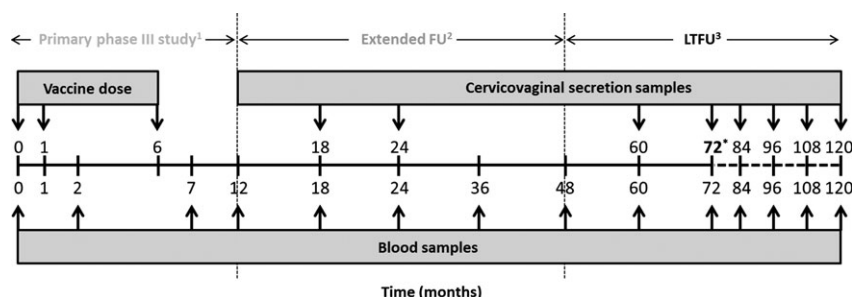

Figure 1. Study design.[1]NCT00196937.[41] [2]Extended follow-up study (NCT00196937).[30] [3]Long-term follow-up study (current study) NCT00947115. *Data presented here are for the year 6 (month 72) time point.

Study vaccine

Each dose of the HPV-16/18 AS04-adjuvanted vaccine contained 20 μg each of HPV–16 and HPV–18 L1 virus-like particles (VLPs) formulated with the AS04 adjuvant system, comprising 50 μg 3–O–desacyl-4'-monophosphoryl lipid A and 500 μg Al(OH)$_3$. The vaccine was supplied in individual 0.5–ml pre-filled syringes and administered into the deltoid muscle.

Study population

Only women who completed the full vaccination course in the primary study were eligible for inclusion in this extended LTFU study. Inclusion and exclusion criteria for the primary study were previously described.[41]

Immunogenicity assessment

Blood samples for immunogenicity assessment were collected at 0, 2, 7, 12, 18, 24, 36, 48, 60 (year 5), and 72 months (year 6), and CVS samples (from women who volunteered) were collected at 18, 24, 60, and 72 months, as previously described.[41] In this LTFU study, we assessed samples collected at year 5 and year 6 (Figure 1). Anti-HPV–16 and anti-HPV–18 antibody titres in serum and CVS samples were measured by ELISA using type-specific VLPs as coating antigens (serum standardised protocol).[5,32,33,41,42] Total immunoglobulin G (IgG) levels were measured to account for variation during the menstrual cycle. IgG titres were evaluated in both serum and CVS samples to standardise the immunogenicity results by dividing each anti-HPV–16 and anti-HPV–18 titre by the corresponding total IgG titre.

Seropositivity was defined as antibody titres ≥ 8 ELISA units (EU)/ml or ≥ 7 EU/ml for anti-HPV–16 and anti-HPV–18, respectively.[30,41] Anti-HPV-16/18 GMTs resulting from natural infection used in this study were previously determined in women aged 15–25 years.[35]

Prediction of long-term persistence of anti-HPV-16/18 antibody responses

Modelling of the long-term persistence of anti-HPV-16/18 antibody responses was assessed as described previously.[43] Briefly, two mixed-effects statistical models, the modified power-law model and the piecewise model, were fitted to the individual anti-HPV-16/18 titres measured at each time point up to year 6 in women vaccinated with the HPV-16/18 AS04-adjuvanted vaccine in the primary study, and for whom post-vaccination results were available. To estimate post-vaccination antibody decay over time, the modified power-law model includes B–cell dynamics, in which two B–cell populations (activated and memory B–cells) are involved, accounting for the long-term persistence of a memory B–cell subpopulation and a long-term antibody plateau. The piecewise model fits the data on three non-overlapping time intervals, corresponding to the observed decay of humoral antibodies. Each piece of the model used a linear function; three break points (7, 12, and 21 months) were selected. The two model effects were fitted using an NLMIXED SAS procedure.

Safety assessment

Although no study vaccine was administered in this LTFU study, all serious adverse events (SAEs) related to vaccination, study participation, or concomitant GlaxoSmithKline Vaccines medication were recorded during the entire study period.

All women with SAEs or who were withdrawn from the study because of SAEs were followed until the SAE had resolved, subsided, stabilised, disappeared, or been otherwise explained.

Statistical analysis

All analyses were performed using SAS 9.2 and PROC STAT-XACT 8.1.

Study cohorts

The primary cohort for analysis of blood sample results at year 6 was the according-to-protocol (ATP) cohort for immunogenicity, which comprised all women included in the ATP immunogenicity analysis in the primary study, meeting all eligibility criteria, complying with procedures defined in the protocol, with no elimination criteria during the study, who attended the year–6 visit, and for whom serology results at year 6 were available.

The primary cohort for analysis of CVS sample results at year 6 was the total vaccinated cohort (TVC), comprising all women who volunteered for CVS sampling at year 6 and for whom CVS results were available at this time point.

The year–6 TVC for safety included all women participating in the current LTFU study. Two safety analyses were performed. The first was based on TVC data collected during this LTFU study from the last visit of the previous follow-up study (up to month 48) to the last visit in this follow-up study (year 6); the second analysis was based on the TVC data collected for the entire study period (from month 0 to year 6).

Immunogenicity analyses

Immunogenicity analyses were age-stratified (15–25, 26–45, and 46–55 years at first vaccination).

Seroconversion was defined as the appearance of antibodies, with titres greater than or equal to the cut-off values, in sera of women who were seronegative before vaccination in the primary study. Conversion for CVS analyses was defined as the appearance of antibodies, with titres greater than or equal to the limits of quantifi-

cation, in CVS samples of women who tested negative before vaccination in the primary study. Seropositivity rates and anti-HPV-16/18 GMTs were calculated with exact 95% confidence intervals (95% CIs). GMT calculations were performed by taking the antilog of the mean of the log titre transformations. In the absence of an accepted serological correlate of protection, we performed descriptive comparisons with anti-HPV-16/18 serology results from a previous efficacy study,[32,33] and with anti-HPV-16/18 titres achieved after natural infection in another study.[35,36]

Safety analyses

Safety analyses were age-stratified (15–25, 26–45, and 46–55 years). Vaccine-, study participation- or concomitant GlaxoSmithKline Vaccines medication-related SAEs and fatal SAEs were described in detail. Vaccine-related SAEs were further evaluated for clinical relevance.

Results

Study population

In the primary study, 666 women received at least one dose of the HPV-16/18 AS04-adjuvanted vaccine. Of the 647 women who received three vaccine doses in the primary study, 159 did not show up for the follow-up visit after 6 years. At year 6, the TVC comprised 488 women, of whom 477 were included in the ATP cohort for immunogenicity (Figure 2). Most women (99.8%) were white/caucasian.

Immunogenicity

Serological immune response

Of the 644 women included in the ATP immunogenicity cohort in the primary study at baseline, over 70% were seronegative for both anti-HPV-16 and anti-HPV-18; among women in the age groups 15–25, 26–45, and 46–55 years, 82.5, 67.4, and 66.3% were seronegative for both HPV–16 and HPV–18, respectively, and only 6% (2.7, 6.9, and 9.0% in the age groups 15–25, 26–45, and 46–55 years, respectively) were seropositive for both HPV–16 and HPV–18.[41] One month following the third vaccine dose (month 7), all initially seronegative women were seropositive for anti-HPV-16/18 antibodies.[41]

At year 6, all women remained seropositive for anti-HPV–16 antibodies and at least 97% were seropositive for anti-HPV–18 antibodies. Four women who were initially seronegative for anti-HPV–18 were still seronegative at year 6 (age group 46–55 years).

Antibody kinetics for both anti-HPV–16 and anti-HPV–18 showed a peak in antibody levels at month 7, followed by a gradual decline until month 18.[35,36] This decline became less pronounced over time, suggesting that a plateau phase had been reached (Figure 3). An age-dependent decrease in GMTs observed at the previous time points was also observed at year 6, as indicated by non-overlapping 95% CIs between the age groups (Figure 3).

At year 6, in initially seronegative women, anti-HPV-16 GMTs were 1344.6 EU/ml (95% CI 1130.2–1599.6 EU/ml), 526.0 EU/ml (95% CI 434.7–636.4 EU/ml), and 277.7 EU/
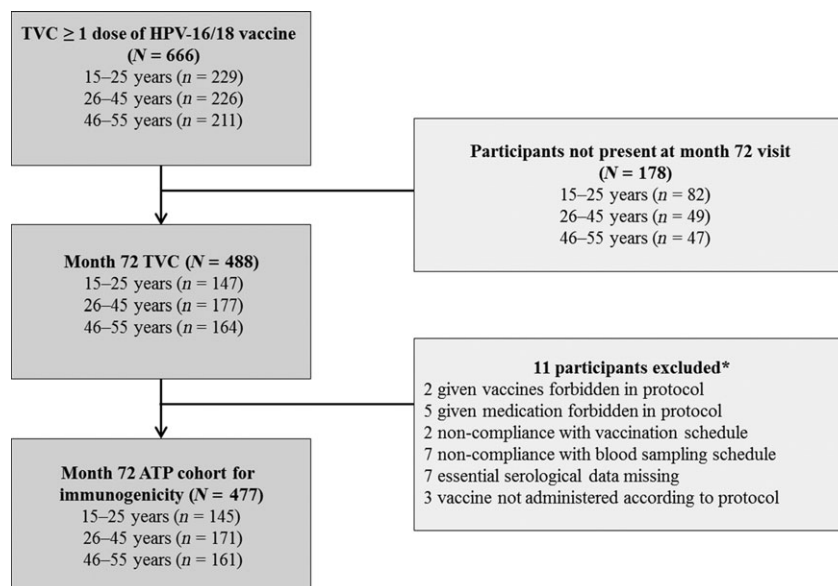

Figure 2. Flow chart of study participants. Key: 15–25 years, women aged 15–25 years at the time of first vaccine dose; 26–45 years, women aged 26–45 years at the time of first vaccine dose; 46–55 years, women aged 46–55 years at the time of first vaccine dose; ATP, according to protocol; N = total number of women; n = number of women in the age group; TVC, total vaccinated cohort. *Participants may have more than one reason for exclusion.

ml (95% CI 228.0–338.2 EU/ml) in the age groups 15–25, 26–45, and 46–55 years, respectively. Anti-HPV–18 GMTs were 438.2 EU/ml (95% CI 366.6–523.7 EU/ml), 167.5 EU/ml (95% CI 138.1–203.1 EU/ml), and 97.6 EU/ml (95% CI 79.2–120.3 EU/ml) in these age groups, respectively. Anti-HPV-16/18 titres were higher than those achieved after natural infection in all age groups: for initially sero-negative women, anti-HPV–16 GMTs were approximately 45.1-, 17.7-, and 9.3-fold higher in the age groups 15–25, 26–45, and 46–55 years, respectively, than the natural infection level (29.8 EU/ml).[35] Anti-HPV–18 GMTs were approximately 19.4-, 7.4-, and 4.3-fold higher in these age groups, respectively, than the natural infection level (22.6 EU/ml).[35,36] At year 6, anti-HPV–16 GMTs were 3.4-fold higher (in the age group 15–25 years) or remained within a similar range (in the age group 26–45 years) when compared with the plateau level established in a previous efficacy study at 45–50 months (397.8 EU/ml).[33] In the age group 46–55 years, anti-HPV–16 GMTs were below the plateau level (0.7–fold; non-overlapping 95% CIs). In the age group 15–25 years, anti-HPV–18 GMTs were also higher (1.5-fold) than the GMT plateau level observed in the previous efficacy study at 45–50 months (297.3 EU/ml).[33] In the age groups 26–45 and 46–55 years, anti-HPV–18 GMTs were lower than this predefined GMT plateau level (0.6-fold and 0.3-fold, respectively), with non-overlapping 95% CIs.

CVS antibody levels (TVC)
At year 6, 190 women had their CVS samples tested, of whom 84 (29 each in the age groups 15–25 and 26–45 years, and 26 in the age group 46–55 years) had CVS samples considered suitable for HPV antibody testing (<200 erythrocytes/μl; samples with $\geq$200 erythrocytes/μl were considered to be contaminated with blood).

At year 6, anti-HPV–16 antibodies were detected in 72.4, 72.4, and 73.1% of CVS samples of women in the age groups 15–25, 26–45, 46–55 years, respectively. At the same time point, anti-HPV–18 antibodies were detected in 69.0, 62.1, and 53.8% of the CVS samples of women in these age
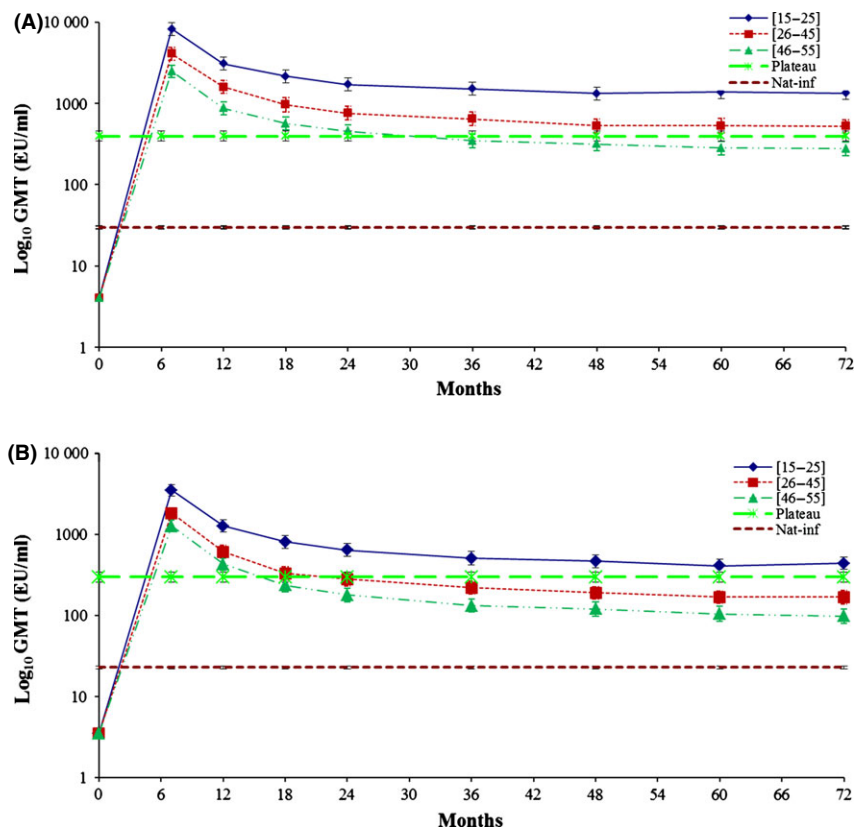

Figure 3. Anti-HPV–16 (A) and anti-HPV–18 (B) antibody levels in initially seronegative women aged 15–55 years (ATP cohort for immunogenicity at year 6). Key: [15–25], women aged 15–25 years at the time of first vaccine dose; [26–45], women aged 26–45 years at the time of first vaccine dose; [46–55], women aged 46–55 years at the time of first vaccine dose; GMT, geometric mean titre; Nat-inf, natural infection. GMTs of women who were (A) HPV–16 or (B) HPV–18 DNA-negative and seropositive at baseline (i.e. who had cleared a natural infection). GMTs were (A) 29.8 EU/ml and (B) 22.6 EU/ml.[35,36] Plateau: GMTs of women aged 15–25 years at months 45–50 after the first vaccine dose (total vaccinated cohort). GMTs were (A) 397.8 EU/ml and (B) 297.3 EU/ml.[33] The error bars represent 95% confidence intervals.

groups, respectively. CVS anti-HPV–16 GMTs were 80.3, 43.8, and 37.1 EU/ml in the age groups 15–25, 26–45, and 46–55 years, respectively, and anti-HPV–18 GMTs were 22.9, 19.9, and 19.2 EU/ml.

At year 6, women with detectable anti-HPV-16/18 antibodies in their CVS samples had higher serum anti-HPV-16/18 GMTs than women with no antibodies detected in their CVS samples: for anti-HPV–16 they were between two- and five-fold higher and for anti-HPV–18 they were between one- and five-fold higher, depending on the age group (data not shown).

To account for the fluctuation in total IgG levels observed during the menstrual cycle, anti-HPV-16/18 antibody levels were standardised relative to the total IgG levels before evaluating the correlation. Correlations between the serum and CVS titres (standardised for total IgG) in the three age groups at year 6 are presented in Figure 4. For anti-HPV–16, the correlation coefficients were high regardless of age, and ranged from 0.87 to 0.91 at year 5,[44] and from 0.81 to 0.96 at year 6 (Figure 4A); for anti-HPV–18, they ranged from 0.84 to 0.87 at year 5,[44] and from 0.69 to 0.84 at year 6 (Figure 4B).

Predicted long-term persistence of antibody responses
The long-term persistence of anti-HPV-16/18 antibody responses was predicted for the three age groups using the modified power-law and piecewise models, based on the 6–year immunogenicity data from the current study. Immunogenicity data obtained at 6.4 years after vaccination in a previous study in women aged 15–25 years were also included in the models (Figure 5).[28] The modified power-law model predicted that mean anti-HPV-16/18 levels would remain above those associated with natural infection for at least 20 years in all age groups. The piecewise model predicted that anti-HPV–16 GMTs would remain above GMTs

associated with natural infection for all age groups, whereas anti-HPV–18 GMTs would remain above the natural infection levels for the age groups 15–25 and 26–45 years, but not for the age group 46–55 years (Table 1). Additional model-based estimations were performed to predict the duration ensuring that 95% of women would have anti-HPV-16/18 levels remaining above the natural infection level. The modified power-law model predicted lifelong anti-HPV–16 levels above the natural infection level for all women, and lifelong anti-HPV–18 levels above the natural infection level in the age groups 15–25 and 26–45 years; for the age group 46–55 years, the modified power-law model predicted that 95% of women would still have anti-HPV–18 levels above natural infection levels after 4.3 years. The piecewise model predicted durations of antibody levels remaining above natural infection levels of up to 40.1, 16.9, and 12.1 years for anti-HPV–16, and up to 19.2, 9.0, and 5.1 years for anti-HPV–18 for 95% of women in the age groups 15–25, 26–45, and 46–55 years, respectively (Table 2).

Safety
The HPV-16/18 AS04-adjuvanted vaccine had a clinically acceptable safety profile in all age groups throughout the entire study duration. Twenty-eight women reported 32 SAEs. One woman (in the age group 26–45 years) reported one SAE (optic neuritis), considered by the investigators as causally related to the study vaccination; as a result of this SAE, the vaccination course of the women was discontinued, but she was not withdrawn from the study. This SAE occurred during the primary study and was resolved therein, as previously reported.[41] Two fatal SAEs occurred during the entire study period: one woman committed suicide and one had a road accident. Both of these SAEs were reported in the age group 46–55 years, and both were considered unrelated to the vaccination.
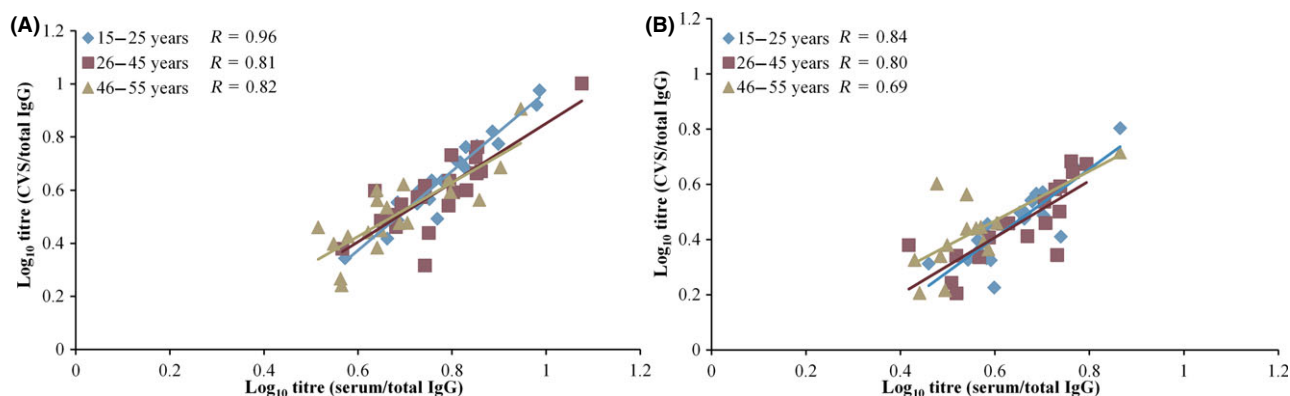

Figure 4. Correlation of ratio between cervicovaginal secretion and serum samples by age for anti-HPV–16 (A) and anti-HPV–18 (B) antibody levels at year 6 in women aged 15–55 years (total vaccinated cohort). Key: [15–25], women aged 15–25 years at the time of first vaccine dose; [26–45], women aged 26–45 years at the time of first vaccine dose; [46–55], women aged 46–55 years at the time of first vaccine dose; CVS, cervicovaginal secretion; R, correlation coefficient. Titres were measured using an ELISA assay for the detection of anti-HPV–16 and anti-HPV–18 antibodies both for cervicovaginal secretion and serum samples. The scatter plots show the ratio (specific IgG/total IgG) transformed to linear log10 values.
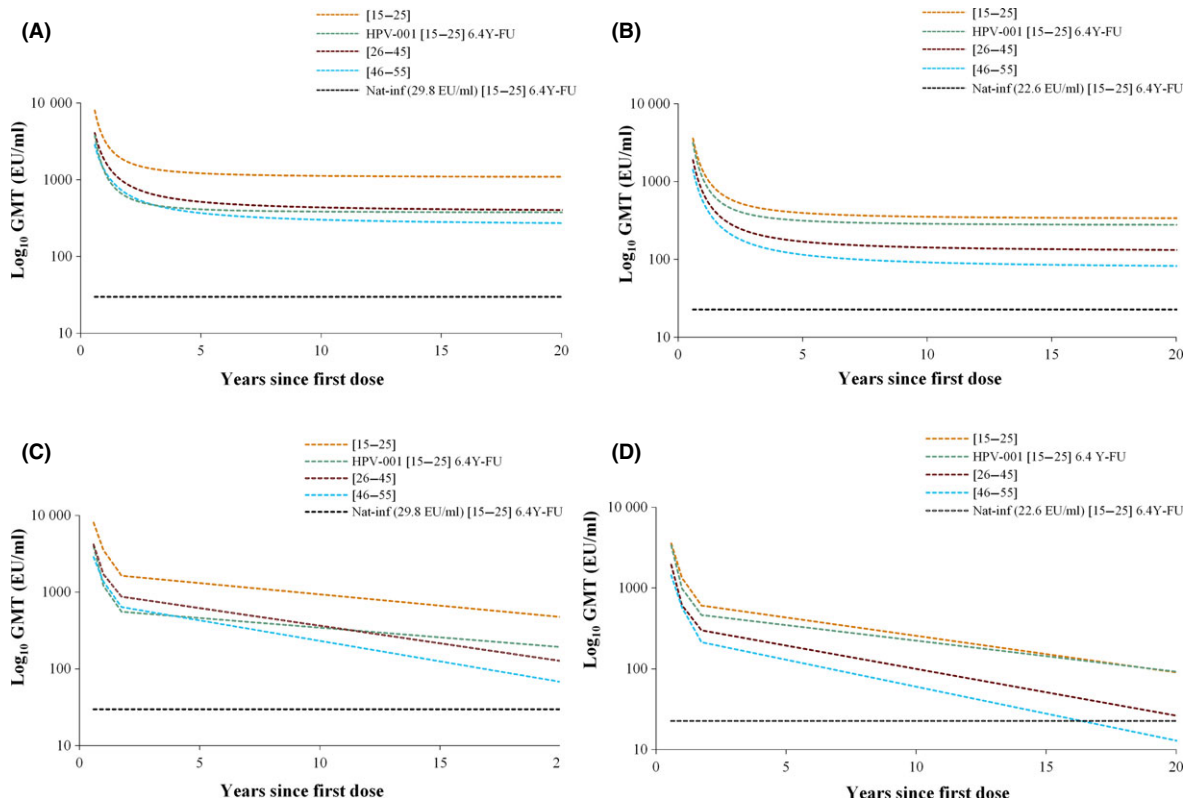
Figure 5. Prediction of antibody responses against HPV–16 and HPV–18 over 20 years using the modified power-law and the piecewise models in women aged 15–55 years (TVC at year 6). (A) Anti-HPV–16 antibody levels prediction by the modified power-law model; (B) anti-HPV–18 antibody levels prediction by the modified power-law model; (C) anti-HPV–16 antibody levels prediction by modified piecewise model; (D) anti-HPV–18 antibody levels prediction by piecewise model. Key: [15–25], women aged 15–25 years at the time of first vaccine dose; [26–45], women aged 26–45 years at the time of first vaccine dose; [46–55], women aged 46–55 years at the time of first vaccine dose; HPV–001 [15–25] 6.4Y–FU, anti-HPV–16 or anti-HPV–18 antibody levels at 6.4 years in women aged 15–25 years from a previous HPV-001 study;[28] Nat-inf, natural infection: geometric mean titres (GMTs) of women who were HPV–16 or HPV–18 DNA-negative and seropositive at baseline (i.e. who had cleared a natural infection). GMTs were 29.8 EU/ml for HPV–16 and 22.6 EU/ml for HPV–18.[35,36]

Table 1. Predicted population GMTs for anti-HPV–16 and anti-HPV–18 at 20 years after the first vaccination in women aged 15–55 years

Antigen	Model	Predicted GMTs 20 years after the first vaccination (EU/ml)*		
		15–25 years**	26–45 years***	46–55 years****
HPV–16	Modified power-law	1092.3	404.2	272.4
	Piecewise	474.9	127.1	67.8
HPV–18	Modified power-law	337.3	132.3	82.2
	Piecewise	90.4	26.4	12.9

*GMTs, geometric mean antibody titres.
**Women aged 15–25 years at the time of first vaccine dose.
***Women aged 26–45 years at the time of first vaccine dose.
****Women aged 46–55 years at the time of first vaccine dose.

Discussion

Main findings

Immune responses to the HPV-16/18 AS04-adjuvanted vaccine persisted up to 6 years after vaccination in women aged 15–55 years. At year 6, all women remained seropositive for anti-HPV-16 and ≥97% were seropositive for anti-HPV-18 antibodies, with anti-HPV-16/18 GMTs higher than levels associated with natural infection; anti-HPV–16 and anti-HPV–18 antibodies were detected

Table 2. Predicted duration ensuring that 95% of women would still have anti-HPV–16 and anti-HPV–18 antibody levels above natural infection levels in women aged 15–25, 26–45, and 46–55 years in the present study, and in women aged 15–25 years in a previous study (NCT00689741 and NCT00120848)[41]

Antigen	Antibody levels induced by natural infection (EU/mL)*	Model	Duration ensuring antibody levels above those induced by natural infection in 95% participants (years)			
			Current study			Previous modelling study**
			15–25 years old***	26–45 years old***	46–55 years old***	15–25 years old***
HPV–16	29.8	Modified power-law	Lifelong	Lifelong	Lifelong	Lifelong
		Piecewise	40.08	16.9	12.1	26.7
HPV–18	22.6	Modified power-law	Lifelong	Lifelong	4.3 years	Lifelong
		Piecewise	19.2	9.0	5.1	19.9

*Measured in a previous study in women who cleared natural infection.[30,31]
**Women aged 15–25 years in a previous study (NCT00689741 and NCT00120848).[41]
***Age ranges indicate the ages of women at the time of the first vaccination dose.

in about 72 and 53.8–69.0% of CVS samples, respectively. A strong correlation between serum and CVS anti-HPV-16/18 GMTs was observed; serum anti-HPV-16/18 GMTs were higher in women with detectable CVS anti-HPV-16/18 antibodies. Exploratory modelling based on the 6–year data predicted that vaccine-induced population anti-HPV-16/18 GMTs would remain above those associated with natural infection for at least 20 years after vaccination. The vaccine had a clinically acceptable safety profile.

Strengths and weaknesses

This study included both young and mature women, allowing for a comparison of anti-HPV-16/18 levels between age groups. Although a sustained HPV-16/18 AS04-adjuvanted vaccine immunogenicity has been reported in women aged 15–25 years,[34,37] to our knowledge this is the first study reporting such long-term (up to 6 years post-vaccination) persistence of antibody responses and correlation of the serum and CVS anti-HPV-16/18 antibodies in women >25 years. Using statistical models, we could for the first time predict the persistence of anti-HPV-16/18 responses for up to 20 years in mature women.

The main study limitations include open design, the lack of a direct control group, and antibody assessment only by ELISA. Assessment of neutralising antibodies, avidity, and cell-mediated immune responses for vaccine and non-vaccine HPV types would provide added value. Another limitation might be the lack of cervical screening data, which could help determine a clinical correlation with the immune data; however, the current study was designed to evaluate the HPV-16/18 AS04-adjuvanted vaccine immunogenicity and safety, and not its efficacy.

Interpretation

We previously showed that the HPV-16/18 AS04-adjuvanted vaccine was immunogenic in women aged 15–55 years for up to 48 months.[30,41] Here, we report the immunogenicity persistence up to 6 years after vaccination. Anti-HPV-16/18 antibody kinetics were similar in all age groups and similar to those reported previously, with a peak response at month 7, followed by a gradual decline.[30,33,41,45] The highest anti-HPV-16/18 levels were observed in the age group 15–25 years, in line with previous observations,[30,41] and consistent with findings that immune responses decline with increasing age.[46]

Although an immunological correlate of protection has not yet been identified, high and sustained antibody titres obtained through vaccination are considered predictive of long-term protection against oncogenic HPV infections and CIN.[47,48] We compared anti-HPV-16/18 levels with GMT plateaus associated with sustained protection at months 45–50 in a previous study on efficacy.[33] In our study, anti-HPV-16/18 levels at year 6 remained above the predefined plateau levels of sustained efficacy in the age group 15–25 years,[33] and in all age groups the levels were substantially higher than the natural infection levels.[35]

A previous modelling study showed the persistence of anti-HPV-16/18 antibody responses up to 20 years following vaccination with the HPV-16/18 AS04-adjuvanted vaccine in women aged 15–25 years.[43] We report for the first time the modelling of antibody responses of up to 20 years after vaccination in older women. Based on our 6–year immunogenicity data, the modified power-law model predicted that post-vaccination anti-HPV-16/18 GMTs would remain substantially higher than levels associated with natural infection for at least 20 years in women aged 15–

55 years. Anti-HPV-16/18 GMTs predicted using the more conservative piecewise model were lower than those predicted with the modified power-law model, but were higher than natural infection levels, except for the age group 46–55 years. Based on immunogenicity data from a previous study (HPV-001),[28] or from the current study for the age groups 15–25 years, both models predicted similar anti-HPV–18 GMTs and lower anti-HPV–16 GMTs 20 years after vaccination. These different predictions may result from the different pre-established assumptions for each model. The modified power-law model is based on the assumption that B–cell memory does not decline over time, whereas the piece-wise model assumes that antibody responses follow a linear decrease over time, and takes into account only antibody levels obtained in a given study. Hence, the piecewise model is likely to yield a more conservative estimate of long-term protection.[43] As the duration of protection associated with HPV vaccination is of crucial importance in cost-effectiveness, our modelling data could serve as a basis to assess the economic impact of cervical cancer vaccination programmes. Finally, a high HPV-16/18 AS04-adjuvanted vaccine efficacy (81.1%, 97.7% CI 52.1–94.0%) against HPV-16/18-related CIN1+ and 6–month persistent infection has been recently demonstrated in women aged ≥26 years.[38] Altogether, these previous results and our immunogenicity data suggest that the HPV-16/18 AS04-adjuvanted vaccine provides a sustained protection against HPV-16/18 infection in women aged >25 years.

A significant relationship between naturally acquired anti-HPV–16 antibodies and the risk of new infection has been reported: the incidence of infection declined with increasing antibody titres.[49] In another study, unvaccinated women with higher anti-HPV-16/18 levels had significantly lower risk of subsequent HPV-16/18 infection than seronegative women, suggesting that natural infection confers a certain degree of protection against new HPV-16/18 infections.[50] Antibody levels after natural infection are much lower than those generated through vaccination, however, and thus natural infection confers only partial protection. The mechanism by which prophylactic HPV vaccines induce protection is assumed to be mediated by vaccine-induced neutralising IgG antibodies, which transudate across cervical epithelium to the HPV infection site and are likely to prevent HPV infection of the cervical basal cell layer at the transformation zone.[47,49,51,52] We observed a strong correlation between the serum and CVS anti-HPV-16/18 levels that persisted up to 6 years after vaccination. To our knowledge, this is the first study showing such long persistence of anti-HPV-16/18 antibody transudation following vaccination with HPV-16/18 AS04-adjuvanted vaccine in women aged >25 years. Although anti-HPV-16/18 levels in serum decreased since the first assessment time point (month 24) in

the primary study,[41] correlation between the serum and CVS antibody levels at year 6 remained high in all age groups. A pooled analysis of data from four clinical trials assessing serum and CVS anti-HPV-16/18 correlation following administration of the HPV-16/18 AS04-adjuvanted vaccine has shown that higher antibody levels in serum resulted in higher antibody levels in CVS, supporting transudation of serum antibodies as the mechanism by which antibodies are introduced into CVS.[51] Furthermore, in a phase–IV clinical trial in girls aged 12–15 years vaccinated with the HPV-16/18 AS04-adjuvanted vaccine, or Gardasil®, cross-neutralising antibodies were detected at the genital site of infection.[53]

The HPV-16/18 AS04-adjuvanted vaccine safety profile was consistent with that described in previous reports.[27,28,31,36]

Conclusion

The HPV-16/18 AS04-adjuvanted vaccine was well tolerated and induced sustained immune responses in women aged 15–55 years. Anti-HPV-16/18 antibody levels remained several-fold higher than levels associated with natural infection for at least 6 years after vaccination. Exploratory modelling of antibody persistence based on the 6–year data predicted that vaccine-induced anti-HPV-16/18 GMTs would remain above those induced by natural infection for at least 20 years, except for anti-HPV–18 levels in the age group 46–55 years (piecewise model). A strong correlation between anti-HPV-16/18 levels in serum and CVS samples 6 years after vaccination indicates a long-lasting transudation of serum antibodies across the cervical epithelium. These results suggest that in addition to the routine vaccination programmes in adolescents, sexually active mature women could potentially also benefit from vaccination with HPV-16/18 AS04-adjuvanted vaccine on an individual basis.

Disclosure of interests

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HPV vaccination: unanswered questions remain

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The development of effective prophylactic vaccines against an infectious agent (human papillomavirus, HPV) that causes cervical cancer is a major milestone in preventive medicine. In this article, Schwarz and co-workers demonstrated persistence for 6 years following immunisation with the Cervarix™ vaccine of high-titer antibodies to HPV–16/18 in the circulation and in cervicovaginal fluid in women aged 15–55 years. Using statistical modelling it was also determined that antibody levels would persist for at least 20 years. An exception was the possible loss of persistent immunity for women aged 46–55 years.

Several questions remain unanswered in relation to the HPV vaccine.

1. Although there is a degree of cross-reactive immunity to other oncogenic HPV types (HPV–31, -33, -45), this cross-protection wanes over time (Malagon et al. *Lancet Infect Dis* 2012;12:781–9). In addition, other oncogenic HPV types (HPV–51, -56) may increase in frequency in vaccinated women (Kavanagh et al. *Br J Cancer* 2014;110:2804–11). Therefore, do vaccinated women need to continue to undergo screening for cervical changes?

2. Are vaccinated women more likely to engage in high-risk sexual behaviour as a result of a false sense of security, compared with their non-vaccinated peers? Are there differences in the rates of sexually transmitted diseases (STDs) and unplanned pregnancies between the two groups? Age-appropriate sex education and screening for STDs should be provided at the time of vaccination.

3. Most women in developed countries have adequate diets and strong immune systems to naturally eliminate HPV should they become infected. They also have access to medical resources to identify and treat early-stage consequences of a persistent HPV infection. The challenge is to provide universal vaccine access to women in resource-poor countries where the incidence of cervical cancer is much higher, and to determine vaccine efficacy and duration under less than adequate environmental conditions. Free or low-cost HPV vaccination programmes, at an age range specific for each region, must be implemented in low-income countries.

4. The mere presence and persistence of antibodies to HPV do not ensure that the antibodies are protective. All women have antibodies to *Candida albicans* but remain susceptible to vulvovaginal candidiasis. The presence and titer of neutralising antibodies must be demonstrated. Does antibody concentration, specificity, or avidity in the genital tract change over time, especially under varying environmental conditions, or in the presence of other genital tract infections. Studies have demonstrated alterations in the vaginal microbiome in HPV-infected women (Gao et al. *BMC Infect Dis* 2013;13:271), and there is an association between elevated vaginal pH and HPV infection (Clarke et al. *BMC Infect Dis* 2012;12:33). There is a need to assess whether the prevalent vaginal disorder, bacterial vaginosis (BV), decreases HPV antibody levels in vaccinated women to non-protective levels, and thereby increases susceptibility to HPV. This would especially be a problem in resource-poor areas where the incidence of BV is usually elevated.

Continued analysis of the efficacy and consequences of HPV vaccination, especially in resource-poor areas, remains a priority.

Disclosure of interests

None. ■