Sustained immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine administered as a two-dose schedule in adolescent girls: Five-year clinical data and modeling predictions from a randomized study

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Abbreviations: 2D, 2-dose; 3D, 3-dose; 95% CI, 95% confidence interval; AS04, Adjuvant System containing 50 μg 3-O-desacyl-4',3'-monophosphoryl lipid A (MPL) adsorbed on aluminum salt (500 μg Al3+); ATV-I, according-to-protocol cohort for immunogenicity; CI, confidence interval; CVT, Costa Rica HPV-16/18 Vaccine Trial; ELISA, enzyme-linked immunosorbent assay; EU, ELISA unit; GMR, ratio of geometric mean antibody titers, GMT(s), geometric mean antibody titer(s); HPV, human papillomavirus; M, month; TVC, total vaccinated cohort; MPL, 3-O-desacyl-4'-monophosphoryl lipid A; VLP(s), virus-like particle(s)

In this randomized, partially-blind study (clinicaltrials.gov; NCT00541970), the licensed formulation of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine (20 μg each of HPV-16/18 antigens) was found highly immunogenic up to 4 y after first vaccination, whether administered as a 2-dose (2D) schedule in girls 9–14 y or 3-dose (3D) schedule in women 15–25 y. This end-of-study analysis extends immunogenicity and safety data until Month (M) 60, and presents antibody persistence predictions estimated by piecewise and modified power law models. Healthy females (age stratified: 9–14, 15–19, 20–25 y) were randomized to receive 2D at M0,6 (N = 240) or 3D at M0,1,6 (N = 239). Here, results are reported for girls 9–14 y (2D) and women 15–25 y (3D). Seropositivity rates, geometric mean titers (by enzyme-linked immunosorbent assay) and geometric mean titer ratios (GMRs; 3D/2D; post-hoc exploratory analysis) were calculated. All subjects seronegative pre-vaccination in the according-to-protocol immunogenicity cohort were seropositive for anti-HPV-16 and 18 (anti-HPV-16: 1.13 [95% confidence interval: 0.82–1.54]; anti-HPV-18: 1.06 [0.74–1.51]). Statistical modeling predicted that in 95% of subjects, antibodies induced by 2D and 3D schedules could persist above natural infection levels for ≥21 y post-vaccination. The vaccine had a clinically acceptable safety profile in both groups. In conclusion, a 2D M0,6 schedule of the HPV-16/18 AS04-adjuvanted vaccine was immunogenic for up to 5 y in 9–14 y-old girls. Statistical modeling predicted that 2D-induced antibodies could persist for longer than 20 y.

Introduction

The human papillomavirus (HPV)-16/18 vaccine (Cervarix®), GSK group of companies has been shown to be immunogenic, efficacious and to have a clinically acceptable safety profile in clinical studies.1-7 The licensed vaccine formulation contains 20 μg of HPV-16 L1 protein virus-like particles (VLPs) and 20 μg of HPV-18 L1 VLPs, formulated with the AS04 Adjuvant System of 3-O-desacyl-4'-monophosphoryl lipid A (MPL; 50 μg) adsorbed on aluminum hydroxide salt (500 μg Al (OH)3). The vaccine was first licensed as a 3-dose (3D) schedule to be given at months (M) 0,1 and 6. However, 3D regimens of
HPV vaccines can be expensive to administer and challenging to complete, particularly in low income countries with limited access to healthcare services.\(^8\) Completion rates for the 3D schedule are also suboptimal in some higher income countries.\(^9\)-\(^12\) Alternative vaccination schedules may improve coverage rates.

Evaluation of 2-dose (2D) schedules of HPV vaccines for preteen/adolescent girls was prompted by the observation that antibody titers to HPV vaccine types following administration of the first vaccine dose.\(^13\),\(^14\) We conducted a Phase I/II study to evaluate the immunogenicity and safety of 2D schedules of the HPV-16/18 AS04-adjuvanted vaccine formulation containing 20 \(\mu g\) each of HPV-16 and –18 L1 virus-like particles and adjuvanted with AS04; 40/40, alternative HPV-16/18 AS04-adjuvanted vaccine formulation containing 40 \(\mu g\) each of HPV-16 and –18 L1 virus-like particles and adjuvanted with AS04; ATP-I, according-to-protocol immunogenicity cohort; M, month; y, years. *Excluding one subject who attended the Month 60 visit but did not sign the informed consent form for this visit. This article focuses on subjects randomized to receive the HPV-16/18 AS04-adjuvanted licensed vaccine formulation (2D 20/20 M0,1,6 and 2D 20/20 M0,6 groups; shaded boxes). Disposition data are also shown for subjects randomized to receive the alternative HPV-16/18 AS04-adjuvanted vaccine formulation (2D 40/40 M0,6 and 2D 40/40 M0,2 groups) for completeness.

**Figure 1.** Flow of participants through the trial. 2D, 2-dose schedule; 3D, 3-dose schedule; 20/20, licensed HPV-16/18 AS04-adjuvanted vaccine formulation containing 20 \(\mu g\) each of HPV-16 and –18 L1 virus-like particles and adjuvanted with AS04; 40/40, alternative HPV-16/18 AS04-adjuvanted vaccine formulation containing 40 \(\mu g\) each of HPV-16 and –18 L1 virus-like particles and adjuvanted with AS04; ATP-I, according-to-protocol immunogenicity cohort; M, month; y, years. *Excluding one subject who attended the Month 60 visit but did not sign the informed consent form for this visit. This article focuses on subjects randomized to receive the HPV-16/18 AS04-adjuvanted licensed vaccine formulation (2D 20/20 M0,1,6 and 2D 20/20 M0,6 groups; shaded boxes). Disposition data are also shown for subjects randomized to receive the alternative HPV-16/18 AS04-adjuvanted vaccine formulation (2D 40/40 M0,6 and 2D 40/40 M0,2 groups) for completeness.
HPV-18 GMTs appeared slightly higher after administration of the 3D schedule than after the 2D schedule.

For the 2D schedule administered to girls aged 9-14 years, vaccine induced anti-HPV-16 and anti-HPV-18 GMTs at Month 60 in the present study were, respectively, 45.9- and 27.6-fold higher than those induced by natural infection\(^1\) and 3.4- and 2.1-fold higher than the corresponding GMTs from the plateau phase (Months 45-50) of a reference study,\(^16\) in which efficacy of the HPV-16/18 AS04-adjuvanted vaccine was demonstrated against HPV-16 and -18 associated infections and histopathological lesions up to 6.4 years after first vaccination in women aged 15-25 years.\(^21\) For the 3D schedule administered to women aged 15-25 years, vaccine induced anti-HPV-16 and anti-HPV-18 GMTs at Month 60 in the present study were, respectively, 48.8- and 28.0-fold higher and 3.7- and 2.1-fold higher than these previously observed in natural infection and plateau benchmarks.

Predicted long-term persistence of antibody responses

Figure 3 depicts the predictions of long-term persistence of HPV-16 and -18 antibody responses for girls aged 9-14 years administered a 2D schedule or women aged 15-25 years administered a 3D schedule (modeled on the basis of 5 years of follow-up data from the current study). Data from 51 of 78 (65.4%) vaccinated girls aged 9-14 years in the 2D group and 95 of 157 (60.5%) of women aged 15-25 years in the 3D group are included.

### Table 1. Summary of demographic characteristics and baseline serostatus by age stratum

| Month 60 TVC | 3D M0,1,6 schedule | 2D M0,6 schedule |
|-------------|---------------------|------------------|
| Age (years) | 12.4 (1.67)         | 12.5 (1.63)      |
| Median      | 13.0                | 13.0             |
| Race, n (%) | 58 (100)            | 109 (98.2)       |
| White - Caucasian / European Heritage | 58 (100) | 107 (98.2) |
| African Heritage / African American | 0 (0.0) | 1 (0.9) |
| American Indian or Alaskan Native | 0 (0.0) | 0 (0.0) |
| Asian - East Asian Heritage | 0 (0.0) | 1 (0.9) |
| Asian - Japanese Heritage | 0 (0.0) | 0 (0.0) |
| Other       | 0 (0.0)             | 0 (0.0)          |
| Month 60 ATP-I | N = 55              | N = 91           |
| HPV-16 baseline serostatus, n (%) | 48 (87.3) | 79 (86.8) |
| Seronegative | 7 (12.7)            | 12 (13.2)        |
| Seropositive | 45 (97.8)           | 74 (87.1)        |
| HPV-18 baseline serostatus, n (%) | 49 (89.1) | 76 (83.5) |
| Seronegative | 10 (19.0)           | 15 (16.5)        |
| Seropositive | 3 (6.5)             | 12 (14.1)        |

2D, 2-dose schedule of the licensed HPV-16/18 AS04-adjuvanted vaccine formulation; 3D, 3-dose schedule of the licensed HPV-16/18 AS04-adjuvanted vaccine formulation; ATP-I, according-to-protocol immunogenicity cohort at Month 60; ELISA, enzyme-linked immunosorbent assay; N, number of subjects in the cohort; n(%); number (percentage) of subjects in the given category; SD, standard deviation; TVC, total vaccinated cohort at Month 60; \(^*\)HPV-16 antibody titer equal to or above the ELISA cut-off of 8 ELISA units/mL pre-vaccination.

### Table 2. Observed HPV-16 and -18 antibody responses by ELISA at Month 60 for initially seronegative subjects in the ATP-I

| Antigen | Statistic | 3D M0,1,6 schedule Women 15-25 years | 2D M0,6 schedule Girls 9-14 years |
|---------|-----------|--------------------------------------|-----------------------------------|
| HPV-16  | N         | 79                                   | 45                                |
|         | Seropositivity rate, n (%) | 79 (100) | 45 (100) |
|         | GMT, EU/mL (95% CI) | 1454.5 (1187.2, 1782.1) | 1369.0 (1104.0, 1697.5) |
|         | GMR (3D/2D) (95% CI) | — | 1.06 (0.78, 1.45) |
| HPV-18  | N         | 76                                   | 43                                |
|         | Seropositivity rate, n (%) | 76 (100) | 43 (100) |
|         | GMT, EU/mL (95% CI) | 634.8 (497.9, 809.3) | 627.2 (476.1, 826.1) |
|         | GMR (3D/2D) (95% CI) | — | 1.01 (0.69, 1.48) |

2D, 2-dose schedule of the licensed HPV-16/18 AS04-adjuvanted vaccine formulation; 3D, 3-dose schedule of the licensed HPV-16/18 AS04-adjuvanted vaccine formulation; 95% CI, exact 95% confidence interval; ATP-I, according-to-protocol immunogenicity cohort; ELISA, enzyme-linked immunosorbent assay; EU/mL, ELISA unit per millilitre; GMR, ratio of geometric mean antibody titers; GMT, geometric mean antibody titer; M, month; N, number of evaluable seronegative subjects in the Month 60 ATP-I; n(%), number (percentage) of seropositive subjects at Month 60.

*Post-hoc exploratory analysis.
As a reference, this figure also depicts predicted GMTs for women aged 15-25 y (Table 3). The modified power law predicts that antibody titers will always remain above those associated with natural infection in 95% of women (i.e., life-long duration) for the 2D schedule administered to girls aged 9-14 y and the 3D schedule administered to women aged 15-25 y (Table 3).

**Safety**

All vaccine formulations and schedules evaluated in this study have been shown previously to have a clinically acceptable reactogenicity and safety profile up to Month 48. In this longer-term evaluation up to Month 60, the safety profile of the licensed formulation of the vaccine was comparable whether administered as a 2D or 3D schedule (Table 4).

Over the 5-year period from Months 0 to 60, 4 pregnancies which ended in spontaneous abortion were reported (1 [3.8%] in the 3D group and 3 [10.0%] in the 2D group) (Table 4). The women were aged 19-27 y at the time of the spontaneous abortion. All four of these pregnancies occurred at least 12 months after the last vaccine dose. The apparent difference in frequencies between groups is likely a chance finding due to the small number of events.

**Discussion**

In this study, we show that 2D of the HPV-16/18 AS04-adjuvanted vaccine administered at 0 and 6 months induced high HPV-16 and -18 antibody responses in girls aged 9-14 y, which are sustained up to 5 y after first vaccination. The kinetics of HPV-16 and -18 antibody responses in the current trial, regardless of whether the vaccine was administered on a 2D or 3D schedule, were similar to those observed in previous clinical trials with this vaccine, with GMTs peaking one month after administration of the last dose and then declining to reach a plateau at approximately 18-24 months after first vaccination. Compared with levels following natural infection, HPV-16 and -18 antibody levels in the vaccine groups were at least 25-fold higher.

Antibody titers were also above those observed at the plateau phase in a previous trial in which 100% vaccine efficacy against CIN2 was demonstrated up to 6.4 y after first vaccination in women aged 15-25 y who received 3D. The inclusion of the AS04 adjuvant system in the vaccine formulation may contribute to the high and sustained antibody titers induced by this vaccine.

HPV-16 and -18 antibody titers elicited by the 2D schedule in girls aged 9-14 y were comparable to those elicited by the 3D schedule in young women aged 15-25 y, with GMT ratios being close to 1 at the 5-year time point. GMT ratios for these 2 groups of subjects have consistently been close to 1 at all previous evaluations, including one month after the last vaccine dose when non-inferiority was formally demonstrated. An appropriately powered Phase III trial of the HPV-16/18 vaccine (NCT01381575) with a larger sample size also demonstrated non-inferiority of HPV-16 and -18 antibody responses for a 2D M0,12 and 2D M0,6 schedule in girls aged 9-14 y compared with the 3D schedule in women aged 15-25 y one and

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**Figure 2.** Observed HPV-16 and -18 geometric mean antibody titers (GMT) and corresponding 95% confidence intervals (CI) by enzyme-linked immunosorbent assay (ELISA) at each time point for subjects in the Month 60 according-to-protocol immunogenicity cohort (ATP-I) who were seronegative at baseline for the corresponding antigen. 2D, 2-dose schedule of the licensed HPV-16/18 AS04-adjuvanted vaccine formulation; 3D, 3-dose schedule of the licensed HPV-16/18 AS04-adjuvanted vaccine formulation. M, month; N, number of evaluable seronegative subjects in the Month 60 ATP-I; Plateau, GMTs at the plateau level (Month 45-50 time point) in women aged 15-25 y administered 3 doses of the HPV-16/18 AS04-adjuvanted vaccine at months 0, 1 and 6 in a previous trial (NCT00120848) were 397.8 and 297.3 ELISA unit (EU)/mL for HPV-16 and -18 antibodies, respectively. Natural infection, GMTs in women aged 15-25 y who had cleared a natural infection in a previous trial (NCT00122681) were 29.8 and 22.7 EU/mL for HPV-16 and -18 antibodies, respectively.1

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As a reference, this figure also depicts predicted GMTs for women aged 15-25 y administered 3 doses of the HPV-16/18 AS04-adjuvanted vaccine at M0,1,6, modeled on the basis of 6.4 y of follow-up data from a previous efficacy study, and GMTs measured in women who had cleared a natural infection in a previous study.1

The piecewise model predicts that HPV-16 and -18 antibody titers will remain above those associated with natural infection in 95% of women for at least 21 y when the vaccine is administered as a 2D schedule to girls aged 9-14 y or a 3D schedule to women aged 15-25 y (Table 3).
6 months after the last vaccine dose, respectively.\textsuperscript{26} Additionally, this larger study showed descriptively similar cross-reacting HPV-31 and −45 antibody titers and cell-mediated immune responses between 2D and 3D groups one month after the last vaccine dose.\textsuperscript{27} On the basis of data from the current study, and the larger Phase III study, a 2D schedule of the HPV-16/18 AS04-adjuvanted vaccine is now approved for girls aged 9–14 y in a number of countries, with flexibility around administration of the second vaccine dose from 5 to 13 months after first vaccination. A 2D schedule at 0 and 6 months of the quadrivalent HPV-6/11/16/18 vaccine is also approved for girls aged 9-13 y. We applied 2 statistical models, using the 5-year data observed in the current study (which included approximately 60% of vaccinated subjects for the relevant age strata in each vaccine group), to predict how long vaccine-induced antibodies are likely to persist. For both models, predictions of persistence were similar for girls aged 9-14 y who received the 2D schedule and women aged 15-25 y who received the 3D schedule, which was expected given the similarity of observed antibody responses over 5 y for these 2 groups. The effect of age upon the magnitude of initial HPV-16 and −18 antibody responses is well documented,\textsuperscript{13,28} but there is no evidence of an interaction between age and time that might indicate different antibody decay rates between age groups.\textsuperscript{29} Using the modified power law, which assumes a progressive decay of antibody and antibody-producing B-cells while assuming that the proportion of memory B-cells remains stable,\textsuperscript{30} lifelong persistence of vaccine-induced antibody titers above those associated with natural infection is predicted for 95% of the population. The piecewise model assumes a linear decay of

| Antigen   | Statistical model          | Predicted GMTs 20 y after first vaccine dose\textsuperscript{*} | Predicted duration of antibody persistence above natural infection levels\textsuperscript{*} in 95% of women |
|-----------|-----------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
|           |                             | 3D M0,1,6 15-25 years 2D M0,6 9-14 years                     | 3D M0,1,6 15-25 years 2D M0,6 9-14 years                                                                 |
| HPV-16    | Piecewise                   | 189.7 EU/mL 157.9 EU/mL                                       | 22.0 years 24.4 years \textsuperscript{3}                                                          |
|           | Modified power law          | 1054.2 EU/mL 1091.0 EU/mL                                     | Always  Always \textsuperscript{3}                                                                |
| HPV-18    | Piecewise                   | 149.1 EU/mL 158.7 EU/mL                                       | 21.5 years 27.3 years \textsuperscript{3}                                                          |
|           | Modified power law          | 497.4 EU/mL 530.3 EU/mL                                       | Always  Always \textsuperscript{3}                                                                |

\textsuperscript{*}For those subjects who received the scheduled number of vaccine doses.

\textsuperscript{3}Natural infection, GMTs in women aged 15-25 y who had cleared a natural infection in a previous trial (NCT00122681) were 29.8 and 22.7 EU/mL for anti-HPV-16 and −18, respectively.\textsuperscript{1} ELISA, enzyme-linked immunosorbent assay.
2D, 2-dose schedule of the licensed HPV-16/18 AS04-adjuvanted vaccine formulation; 3D, 3-dose schedule of the licensed HPV-16/18 AS04-adjuvanted vaccine formulation; 95% CI, exact 95% confidence interval. New events occurring since the previous reporting period for this trial.

Using the piecewise model, it is predicted that vaccine-induced antibody titers will be sustained above those associated with natural infection for at least 21 y for 95% of the population.

This study was not designed to assess efficacy, but using the principle of immunobridging we infer that protection against HPV infection and cervical disease in adolescent girls given a 2D schedule will be similar to that previously observed in women aged 15-25 y given a 3D schedule. The large Phase III efficacy trial (PATRICIA) conducted with a 3D schedule of the HPV-16/18 AS04-adjuvanted vaccine in women aged 15-25 y showed very high vaccine efficacy against grade 3 or 4 cervical intraepithelial neoplasia in HPV-naive women, irrespective of HPV type in the lesion. Proof-of-principle for the efficacy of fewer than 3 vaccine doses comes from a post-hoc analysis of the Costa Rica HPV-16/18 Vaccine Trial (CVT), conducted in women aged 18-25 y. While this comparison was not randomized, similar vaccine efficacy was observed against 12-month persistent HPV-16 and -18-associated infection in women who received only 2 or even one dose of the 3 scheduled vaccine doses compared with those women who received all 3 doses. Immunological evaluation from the CVT showed that both anti-HPV-16 and -18 GMTs (by ELISA) among women who received 2 vaccine doses separated by 6 months were non-inferior to those in women who received the complete 3D schedule 4 y after first vaccination and that anti-HPV-16 and -18 GMTs in women who received 2 vaccine doses were at least 24- and 14-fold higher than those observed in natural infection.

We previously showed that both the 2D and 3D schedules had a clinically acceptable reactogenicity profile in this study, and no safety concerns were raised during this 5-year follow-up. Safety findings were generally in accordance with a pooled analysis of data from completed or ongoing clinical studies of the HPV-16/18 AS04-adjuvanted vaccine, which show that it has an acceptable benefit-risk profile in adolescent girls and adult women.

A strength of our study is that this is the longest period of follow-up for a 2D schedule of an HPV vaccine, providing confidence in the persistence of responses with a reduced dose schedule. A limitation is that the study was designed and powered to evaluate non-inferiority of antibody responses at Month 7 only. Results from exploratory comparisons of GMT ratios at subsequent time points should be interpreted with caution as there was no adjustment for multiplicity and the clinical relevance of any difference was not accounted for in the planning of the exploratory analysis. Although the statistical modeling predicts that administration of a 2D schedule of the HPV-16/18 AS04-adjuvanted vaccine to preteen/adolescent girls will provide long-term persistence of HPV-16 and -18 antibodies, which may protect them from the consequences of HPV-16 and -18 infection for most of their sexually active lives, these conclusions can only be considered as informative until long-term observational data are available. The study was conducted in healthy females of predominantly Caucasian ethnicity, and it is not known if the predictions regarding long-term antibody kinetics can be extrapolated to other populations.

In conclusion, the durable immune response elicited by a 2D schedule of the HPV-16/18 vaccine in preteen/adolescent girls is...
predicted to provide long-lasting protection against HPV infection and subsequent development of high-grade cervical lesions and cancer. A 2D schedule is likely to offer logistical and economic advantages over a 3D schedule, which in turn may facilitate greater vaccination coverage rates for adolescent girls, with the potential to substantially reduce the global burden of cervical cancer.

Methods and Participants

Study design, participants and ethics

The design of this Phase I/II, partially-blind, controlled, randomized, parallel group trial has been reported previously. The study was conducted at 21 centers in Canada and Germany. It was initiated in October 2007 and data for the Month 60 analysis were collected up to March 2013. Briefly, healthy girls and young women aged 9-25 y at the time of first vaccination in the study were stratified by age (9-14, 15-19, 20-25 y) and randomized (1:1:1:1) to receive 3 doses of the licensed HPV-16/18 AS04-adjuvanted vaccine formulation (containing 20 μg each of HPV-16 and −18 L1 VLPs adjuvanted with AS04) at 0, 1 and 6 months, 2 doses of the licensed vaccine formulation at 0 and 6 months, 2 doses of an alternative vaccine formulation (containing 40 μg each of HPV-16 and −18 L1 VLPs adjuvanted with AS04) at 0 and 6 months, or 2 doses of the alternative vaccine formulation at 0 and 2 months.

The trial was approved by the appropriate Independent Ethics Committee for each center and was conducted according to the Declaration of Helsinki and Good Clinical Practice. The trial is registered with www.clinicaltrials.gov (registration number NCT00541970). A summary of the protocol is available at www.gsk-clinicalstudyregister.com (GSK study ID 110659). All participants provided written informed consent, or informed assent with written consent from a parent or legal representative (if below the legal age of consent).

Study data up to 4 y after first vaccination (including primary and secondary endpoints) have been published previously. Here, we extend immunogenicity and safety data through 5 y after first vaccination and present statistical modeling predictions of antibody persistence. We focus on results for girls aged 9–14 y who received the 2D schedule of the licensed vaccine formulation and women aged 15–25 y who received the 3D schedule of the licensed vaccine formulation.

Vaccines, randomization and masking

Each 0.5 mL dose of the licensed vaccine formulation (Cervarix®, GSK group of companies) contained 20 μg of HPV-16 and 20 μg of HPV-18 L1 VLPs adjuvanted with AS04 and each 0.5 mL vaccine dose of the alternative vaccine formulation contained 40 μg of HPV-16 and 40 μg of HPV-18 L1 VLPs adjuvanted with AS04. AS04 is a GSK proprietary Adjuvant System containing MPL (50 μg) adsorbed on aluminum hydroxide salt (500 μg Al(OH)₃). Vaccine doses were administered by intramuscular injection in the deltoid region of the non-dominant arm.

The randomization schedule was generated by GSK Vaccines using validated software. The study was observer-blind within the 2D schedule groups, with blinding maintained to Month 7, as reported previously. The study was open within the 3D group.

Immunological evaluation

Blood samples for serologic evaluation were drawn prior to first vaccination (Month 0), at Month 3 (2D groups only), and at Months 7, 12, 18, 24, 36, 48 and 60. Antibodies to HPV-16 and −18 were measured by ELISA, as described previously. Seropositivity was defined as an antibody titer greater than the assay cut-off. For time points from Month 0 through Month 48, the assay cut-off was 8 ELISA unit (EU)/mL for HPV-16 and 7 EU/mL for HPV-18. The assay used to measure HPV-16 and −18 antibody concentrations at the designated laboratory was recently improved to increase precision, consequently for the Month 60 time point the assay cut-off changed from 8 EU/mL to 19 EU/mL for HPV-16 and from 7 EU/mL to 18 EU/mL for HPV-18.

Safety evaluation

Serious adverse events, adverse events leading to withdrawal, other medically significant conditions (ie, adverse events prompting emergency room or physician visits that were not related to common diseases), new onset chronic diseases including new onset autoimmune diseases and pregnancies occurring through Month 60 were documented. Pregnancies were followed until delivery. As described previously, all adverse events reported during the trial were compared with a pre-defined list of potential chronic diseases derived from the Medical Dictionary for Regulatory Activities. Determination of whether a chronic disease was of new onset was based on blinded review of the reported symptoms and the subject’s pre-vaccination medical history by a physician from GSK. A separate list, restricted to potential autoimmune events which excluded allergy-related events or isolated signs and symptoms and events not considered to be autoimmune in origin, was used to identify new onset autoimmune diseases among events identified as new onset chronic diseases.

Statistical methods

The sample size justification for this study has been reported previously. The TVC included all vaccinated subjects and the Month 60 TVC included all vaccinated subjects with data at Month 60. The Month 60 ATP-I included all evaluable subjects (ie, those meeting all eligibility criteria, complying with the procedures defined in the protocol, with no elimination criteria during the trial) for whom data concerning immunogenicity endpoints were available. This included subjects who returned for blood sampling at Month 60 and for whom assay results were available for antibodies against at least one study vaccine antigen component after vaccination. Analyses were performed using SAS 9.2 and PROC StatXact 8.1.

Seroconversion and seropositivity rates with exact 95% confidence interval (CI) and GMTs (with 95% CI) for HPV-16 and −18 antibodies were calculated by pre-vaccination serostatus.
GMTs were computed by taking the anti-log of the mean of the log titer transformations; antibody titers below the cut-off of the assay were given an arbitrary value of half the cut-off in this calculation. HPV-16 and −18 antibody GMTs in the present study were compared descriptively with those observed in women aged 15–25 y in a previous study who had cleared a natural infection (29.8 and 22.7 EU/mL, respectively), as well as with those measured at the plateau phase at Months 45–50 in another study (397.8 and 297.3 EU/mL, respectively), in which vaccine efficacy was demonstrated in women aged 15–25 y. The proportion of participants with at least one report of a serious adverse event, medically significant condition, new onset chronic disease, and new onset autoimmune disease were calculated with exact 95% CI.

In a post-hoc exploratory analysis, anti-HPV-16 and −18 titers at Month 60 were compared between 2D and 3D schedules for initially seronegative subjects in the Month 60 ATP-1 by calculating the ratio of GMTs with exact 95% CI (3D schedule in women aged 15-25 y divided by the 2D schedule in girls aged 9-14 y).

In post-hoc exploratory analyses of the persistence of vaccine-induced antibodies, 2 different mixed effects models (the modified power law and the piecewise models) were fitted to the individual HPV-16 and −18 antibody titers measured at each time point up to Month 60 in participants in the total vaccinated cohort who had received the scheduled number of doses of the HPV-16/18 AS04-adjuvanted vaccine and for whom results were available at all the post-vaccination time points, as previously described. The piecewise model fitted the data on 3 non-overlapping time intervals, corresponding to the observed decay of humoral antibodies. Each piece of the model used a linear function, and 3 break points (months 7, 12 and 21) were selected on the basis of Akaike's Information Criterion. The modified power law model includes B-cell dynamics to estimate antibody decay over time after vaccination, in which 2 populations of B-cells (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 and power law models were fitted using, respectively, a MIXED and a NLMIXED SAS procedure.

Disclosure of Potential Conflicts of Interest

B.R. received grants, travel support and speaker’s fees through her institution and her Professional Corporation from the GSK group of companies outside of the submitted work. L.F. has received honoraria from the GSK group of companies during the conduct of the study and personal fees from the GSK group of companies outside of the submitted work. K.P. received fees from the GSK group of companies to conduct the study. K.S. received payments from the GSK group of companies during the conduct of the study and outside of the submitted work. The institution of P.H. received grants/grants pending from the GSK group of companies during the conduct of the study and outside of the submitted work. P.S., F.T. and F.S. are employees of the GSK group of companies. F.T. and F.S. also own restricted shares/stock options in the GSK group of companies. M.D. and U.B. have nothing to disclose.

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Authors’ Contributions

T.S. was one of the coordinating investigators and together with B.R., L.F., K.P., M.D., U.B., K.S. and P.H. participated in the recruitment and/or follow-up of subjects. T.S. designed the study in collaboration with GSK Vaccines. At GlaxoSmithKline (India), P.S. contributed toward data analyses and interpretation, and prepared the statistical analysis report. F.S. and F.T. supervised the conduct of the study at GSK Vaccines (Belgium), and together with T.S. critically reviewed the study report. All authors reviewed and commented upon the drafts of the manuscript and gave final approval to submit for publication. All authors had full access to the data. The authors received no financial support or other form of compensation for the development of the manuscript.
Cervarix is a registered trade mark of the GSK group of companies.

Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.
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