Sustained efficacy and immunogenicity of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine: analysis of a randomised placebo-controlled trial up to 6·4 years

*The GlaxoSmithKline Vaccine HPV-007 Study Group**

Summary

Background Prophylactic human papillomavirus (HPV) vaccines have to provide sustained protection. We assessed efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine up to 6·4 years.

Methods Women aged 15–25 years, with normal cervical cytology, who were HPV-16/18 seronegative and oncogenic HPV DNA-negative (14 types) at screening participated in a double-blind, randomised, placebo-controlled initial study (n=1113; 560 vaccine group *vs* 553 placebo group) and follow-up study (n=776; 393 *vs* 383). 27 sites in three countries participated in the follow-up study. Cervical samples were tested every 6 months for HPV DNA. Management of abnormal cytologies was prespecified, and HPV-16/18 antibody titres were assessed. The primary objective was to assess long-term vaccine efficacy in the prevention of incident cervical infection with HPV 16 or HPV 18, or both. We report the analyses up to 6·4 years of this follow-up study and combined with the initial study. For the primary endpoint, the efficacy analysis was done in the according-to-protocol (ATP) cohort; the analysis of cervical intraepithelial neoplasia grade 2 and above (CIN2+) was done in the total vaccinated cohort (TVC). The study is registered with ClinicalTrials.gov, number NCT00120848.

Findings For the combined analysis of the initial and follow-up studies, the ATP efficacy cohort included 465 women in the vaccine group and 454 in the placebo group; the TVC included 560 women in the vaccine group and 553 in the placebo group. Vaccine efficacy against incident infection with HPV 16/18 was 95·3% (95% CI 87·4–98·7) and against 12-month persistent infection was 100% (81·8–100). Vaccine efficacy against CIN2+ was 100% (51·3–100) for lesions associated with HPV-16/18 and 71·9% (20·6–91·9) for lesions independent of HPV DNA. Antibody concentrations by ELISA remained 12-fold or more higher than after natural infection (both antigens). Safety outcomes were similar between groups: during the follow-up study, 30 (8%) participants reported a serious adverse event in the vaccine group versus 37 (10%) in the placebo group. None was judged related or possibly related to vaccination, and no deaths occurred.

Interpretation Our findings show excellent long-term efficacy, high and sustained immunogenicity, and favourable safety of the HPV-16/18 AS04-adjuvanted vaccine up to 6·4 years.

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Introduction

Cervical cancer is the second most common malignant disease in women worldwide, with the largest burden in developing countries.[1] In 2002, there were nearly 500 000 new cases of cervical cancer and 270 000 deaths from the disease.[1] Cervical cancer has substantial societal effects, since it affects women at a younger age than do most other cancers.[2]

Infection with oncogenic human papillomavirus (HPV) is the necessary cause of cervical cancer.[3] 15 oncogenic HPV types have been identified,[4] most belonging to two important families: types related to HPV 16 and those related to HPV 18.[5] Studies of HPV distribution in invasive cervical cancer have shown a prevalence of about 70% for HPV 16 and HPV 18 combined, with other common types being HPV 31, 33, 45, 52, and 58.[6,7] This distribution seems to be similar across continents. There is a larger contribution of HPV 18 and HPV 45 in adenocarcinoma than in squamous-cell carcinoma.[7]

Prophylactic vaccines against HPV infection are expected to provide a major advance in the prevention of cervical cancer. Such vaccines have to provide long-term protection, since the risk of acquiring an infection starts at sexual debut and women remain vulnerable to development of HPV-related lesions throughout their life. Serum neutralising antibodies are believed to be a major basis of protection offered by HPV vaccines.[8,9] Antibody concentrations after natural infection are low.[10] Women with a naturally acquired HPV infection remain at risk for a new infection with the same HPV type, possibly because antibody concentrations after natural infection are insufficient to confer protection.[11,12] Alternatively, these results could be due to inadequate antibody assay specificity. In one

study, a sustained high concentration of IgG antibody to HPV 16 after natural infection was associated with a reduced risk of subsequent infection with HPV 16 and related types, whereas individuals with a low concentration of IgG antibody were not protected.[13] Therefore, in the absence of a serological correlate of protection, which has yet to be identified, vaccination should induce higher neutralising antibody concentrations than should natural infection.[14,15]

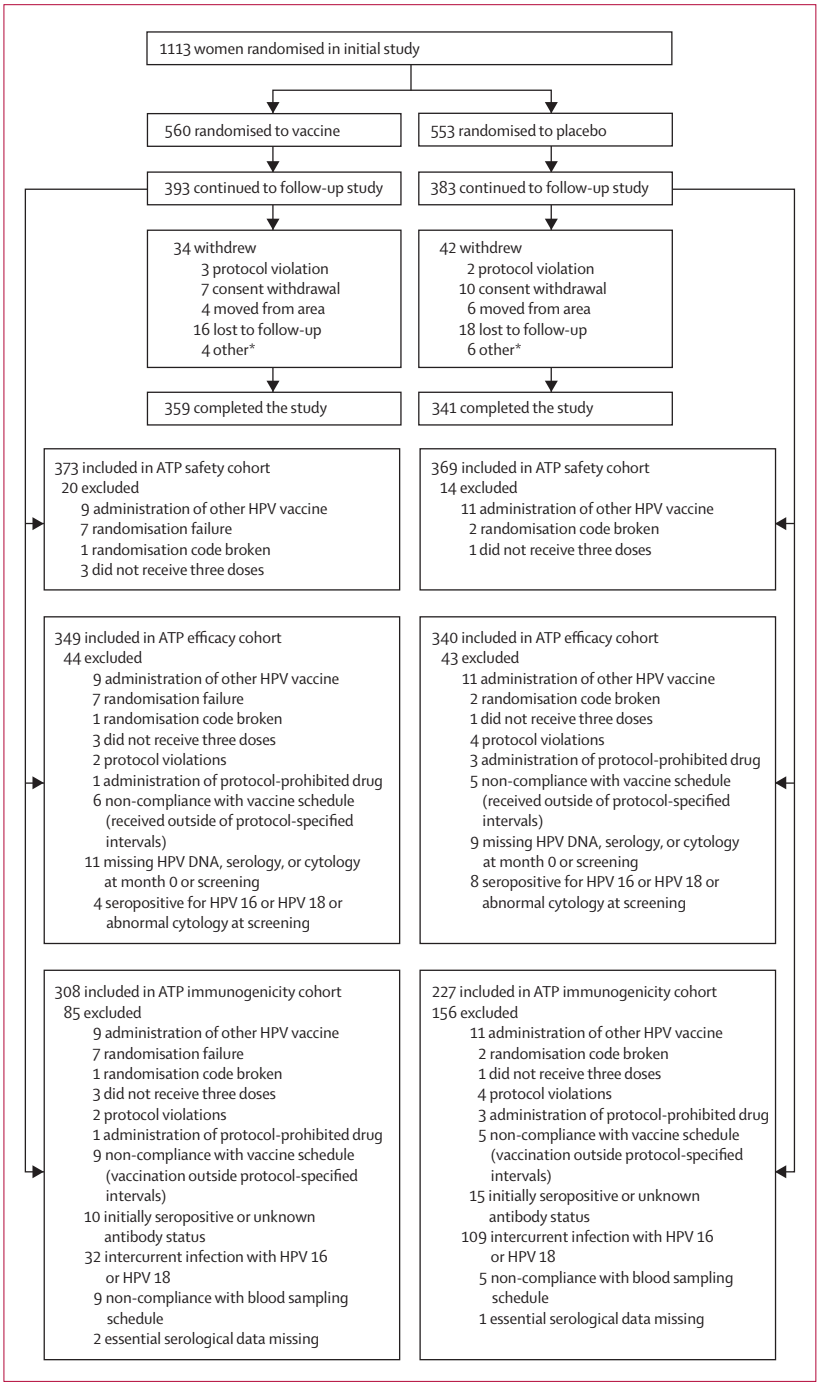

Figure 1: **Disposition of participants**
Some women were excluded from the according-to-protocol (ATP) cohorts during the initial study. *Mainly pregnancy and inability to attend visits.

The HPV-16/18 vaccine is adjuvanted with AS04—an adjuvant system comprising aluminium salt and the immunostimulatory molecule, 3-O-desacyl-4′-monophosphoryl lipid A (MPL).[16] Studies of AS04-adjuvanted vaccines have shown that they produce consistently higher antibody titres that are sustained over a longer period, together with a higher frequency of memory B cells, than do the same antigens adjuvanted with aluminium salts alone.[17,18]

Findings from several clinical studies[19–23] have shown that the HPV-16/18 AS04-adjuvanted vaccine has a strong and sustained antibody response and a favourable safety profile. The initial efficacy study started in 2001 and the long-term follow-up study in 2003. Results of the initial study and of two interim analyses of the follow-up study have been reported previously.[21,22,24] Here, we report the analysis of the follow-up study, with a total follow-up of up to 6·4 years after vaccination.

Methods

Study setting, design, and participants

The method of the initial study[21] and the follow-up study[22] has been described previously. In brief, the follow-up study took place in 27 sites (five in Brazil, five in Canada, and 17 in the USA) between Nov 10, 2003, and Aug 9, 2007.[21,22] The study protocol, which described the length of follow-up and all prespecified timepoints for analysis, and written informed consent, which was obtained at the screening visit from all participants or from a legally acceptable representative for those younger than the legal age of consent, were approved by independent ethics committees or institutional review boards.

Healthy young women (aged 15–25 years) who had normal cervical cytology and were HPV-16 and HPV-18 seronegative by ELISA and HPV DNA-negative by PCR for 14 oncogenic HPV types in exocervical cells at screening were enrolled in the double-blind, multicentre, randomised, placebo-controlled initial study. The HPV-16/18 AS04-adjuvanted vaccine (GlaxoSmithKline Biologicals, Rixensart, Belgium) and placebo, and their administration, have been described previously.[21,22]

In the initial study, women were followed up to at least month 18. Women who received all three doses of study vaccine or placebo and for whom treatment allocation had remained masked were eligible for the 3-year follow-up study, which included seven scheduled visits. Treatment allocation remained masked throughout initial and follow-up studies. In total, including the initial study and the follow-up study, women were followed for up to 6·4 years after the first vaccine dose. Although the main focus of this Article is on analysis up to 6·4 years, key efficacy outcomes from the second interim analysis (up to 5·5 years after vaccination) that have not been previously published are also presented.

Procedures

The primary objective was to assess long-term vaccine efficacy in the prevention of incident cervical infection with HPV 16 or HPV 18, or both, in adolescent and young adult women. Secondary objectives included long-term vaccine safety and immunogenicity, and long-term vaccine efficacy against incident and persistent infection and cytological and histopathological abnormal changes associated with oncogenic HPV types.

Cervical samples were collected every 6 months, and a broad range PCR SPF_{10}-$LiPA_{25}$ system was used to detect 14 oncogenic HPV types and 11 non-oncogenic HPV types; positive specimens were tested by line probe assay and type-specific HPV-16 and HPV-18 PCR as previously described.[21,22] Incident and 6-month and 12-month persistent infection were defined as previously described,[22] and vaccine efficacy calculated.

Collection of cytology and histopathology specimens per management and colposcopy referral guidance has been previously described.[21,22] Vaccine efficacy against cytological abnormal changes (atypical squamous cells of undetermined significance [ASC-US] or greater, low-grade squamous intraepithelial lesions [LSIL] or greater) and histopathologically-confirmed cervical intraepithelial neoplasia grade 1 and above (CIN1+) and grade 2 and above (CIN2+) associated with oncogenic HPV types was assessed. CIN1+ was defined as CIN grades 1–3, adenocarcinoma in situ, and invasive carcinoma; CIN2+ excluded CIN1. Typing of HPV DNA present in the cytology sample or lesion was done by PCR as previously described.[21,22] HPV DNA detected in the samples was considered to be associated with the clinical endpoint (prespecified analysis).

Total IgG antibody titres to HPV 16 and HPV 18 were measured by ELISA as previously described.[21,22] Neutralising antibody titres to HPV 16 and HPV 18 were also assessed with the pseudovirion-based neutralisation assay.[25] This test was done in a protocol-defined subset of 150 participants who were randomly selected by the external statistician for the final analysis.

Blood samples were taken at months 0, 7, 12, and 18 during the initial study and on a yearly basis during the follow-up study. Geometric mean titres (GMT) with 95% CIs were calculated. Because women were enrolled into the follow-up study independently of the date of their first vaccination, results in the follow-up study are allocated to time intervals (covering a 6-month period) relative to first vaccine administration. Immunogenicity data are reported for up to 76 months after first vaccination.

Serious adverse events, adverse events, including new onset chronic diseases, and pregnancy outcomes were assessed throughout the follow-up study. A serious adverse event was defined as an event that resulted in death, was life-threatening, or needed prolonged admission to hospital, resulted in disability or incapacity, was a congenital abnormality or birth defect in the child of a study participant, or other important medical events in the judgment of the investigator. A new onset chronic disease included, for example, diabetes mellitus and autoimmune diseases.

Statistical analysis

Efficacy results are presented for a combined analysis of the initial study and the follow-up study, and immunogenicity data are shown from the initial and follow-up studies. Safety results for the initial study have been extensively described previously.[21] This Article presents long-term safety data from the follow-up study. The study remains masked for individual participants, since a subset of women is continuing follow-up in a separate study.

	HPV-16/18 AS04-adjuvanted vaccine		Placebo		Vaccine efficacy (%; 95% CI)
	Total number of women	Women reporting ≥1 event	Total number of women	Women reporting ≥1 event	
ATP efficacy cohort					
Incident infection	401	4	372	70	95·3% (87·4–98·7)
6-month persistent infection	401	0	372	34	100% (90·0–100)
12-month persistent infection	401	0	372	20	100% (81·8–100)
TVC					
≥ASC-US	505	2	497	54	96·7% (87·3–99·6)
≥LSIL	505	2	497	34	94·6% (78·8–99·4)
CIN1+	481	0	470	15	100% (73·4–100)
CIN2+	481	0	470	9	100% (51·3–100)

Combined analysis of the initial and follow-up studies. According-to-protocol (ATP) efficacy cohort includes women who met all eligibility criteria, complied with study procedures, and had data available for efficacy measures. Total vaccinated cohort (TVC) includes women who received at least one dose of study vaccine or placebo and for whom endpoint measures were available; this cohort was previously described[21,22] as an intention-to-treat cohort. All infections, cytology results, and lesions are associated with infection with either HPV 16 or HPV 18, or both. 6-month persistent infection: detection of the same HPV type in two consecutive samples over a minimum of 5 months. 12-month persistent infection: detection of the same HPV type in two consecutive samples over a minimum of 10 months. ASC-US=atypical squamous cells of undetermined significance or greater. LSIL=low-grade squamous intraepithelial lesions or greater. CIN1+=cervical intraepithelial neoplasia grade 1 and above. CIN2+=cervical intraepithelial neoplasia grade 2 and above.

Table 1: **Vaccine efficacy against HPV-16/18 incident and persistent infection in cervical samples and HPV-16/18 associated cytological and histopathological endpoints up to 6·4 years of follow-up**

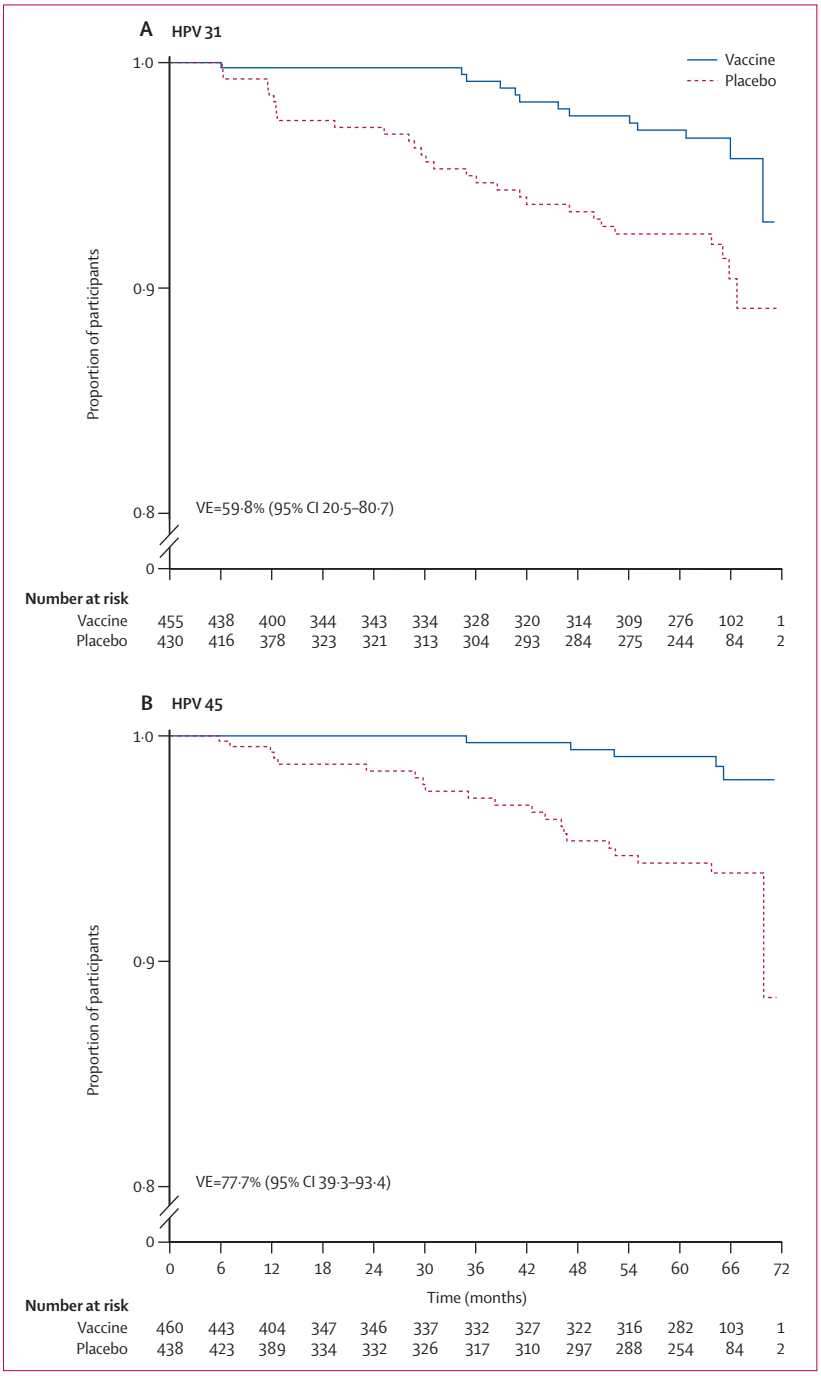

Figure 2: **Reverse cumulative distribution curves for incident infection with HPV 31 (A) and HPV 45 (B) in cervical samples**

Combined analysis of initial and follow-up studies in according-to-protocol efficacy cohort. VE=vaccine efficacy.

Primary analyses of efficacy were done on the according-to-protocol (ATP) cohort for efficacy for virological endpoints (incident and persistent infection) and on the total vaccinated cohort (TVC) for efficacy for cytohistological endpoints. Primary analyses of immunogenicity and safety were done on the ATP cohorts for immunogenicity and safety, respectively. Pregnancy outcomes are reported for the TVC. The ATP immunogenicity and efficacy cohorts included women who met all eligibility criteria, complied with study procedures, and had data available for at least one vaccine antibody blood sample (ATP immunogenicity cohort) or data available for the efficacy measure considered (ATP efficacy cohort). The ATP safety cohort included all assessable women who did not use any investigational or non-registered product or any HPV vaccine other than study vaccine during the study period. For the combined analysis of the initial and follow-up studies, the TVC included women who received at least one dose of study vaccine or placebo and for whom endpoint measures were available. In previous publications of this study, we referred to an intention-to-treat (ITT) cohort.[21,22] However, since studies of preventive vaccines commonly report results with a TVC analysis, we have used this term throughout the Article. In fact, all women enrolled in the initial study were randomly assigned and received at least one dose of vaccine or placebo correctly in accordance with the randomisation schedule,[21] and thus the ITT cohort and the TVC are identical in this study. Women were not included in immunogenicity assessments if HPV infection was detected for the type under consideration during the study periods to exclude any effect of a natural infection on the immune response. Women were censored from efficacy assessments once a specific endpoint was met.

Details of the statistical analysis have been reported previously.[22] Analysis of the combined results of the initial study and the follow-up study was descriptive. We did post-hoc analyses of vaccine efficacy against ASC-US or greater and LSIL or greater, and overall efficacy on cytological and histopathological endpoints irrespective of HPV DNA results. We used the conditional exact method to estimate vaccine efficacy as described previously.[22]

This study is registered with ClinicalTrials.gov, number NCT00120848.

Role of the funding source

Investigators from the GlaxoSmithKline HPV-007 Study Group obtained data from the study and cared for the participants. All statistical testing was done by external, independent statisticians to maintain masking. The GlaxoSmithKline HPV-007 Study Group had access to the study report and had final responsibility for the decision to submit for publication.

Results

Of the 1113 women included in the initial study, 776 continued in the follow-up study, which 700 (90%) completed. Figure 1 shows the disposition of the participants in the follow-up study. For the combined analysis of the initial and follow-up studies, the TVC included 560 women in the vaccine group and 553 in the

placebo group, whereas the ATP efficacy cohort included 465 in the vaccine group and 454 in the placebo group.

The mean follow-up period from the start of the initial study to the end of the follow-up study was 5·9 years (SD 0·3), with a maximum duration of 6·4 years. The demographic profile of participants was similar in both groups (data not shown). The study population was racially diverse with a mean age of 23 years (range 17–29; SD 3) at entry into the follow-up study (mean age at entry into the initial study was 20 years [range 15–26; SD 3]). The demographic characteristics at enrolment in the initial study (age, height, weight, and ethnic origin) were similar for the women who entered the initial study and the subset who enrolled in the follow-up study (data not shown).

Vaccine efficacy remained very high against incident HPV-16 or HPV-18 infection, or both, up to 6·4 years (table 1). Long-term vaccine efficacy against persistent infection for HPV 16 or HPV 18, or both, was 100% for the 6-month and 12-month definition (table 1).

13 women in the vaccine group had an incident infection with HPV 31 compared with 30 in the placebo

	HPV-16/18-AS04 adjuvanted vaccine		Placebo		Vaccine efficacy (%; 95% CI)
	Total number of women	Women reporting ≥1 event	Total number of women	Women reporting ≥1 event	
Incident infection with HPV 16/18					
2·2 years	366	2	355	23	91·6% (64·5 to 98·0)
4·5 years	414	3	385	51	94·7% (83·5 to 98·9)
5·5 years	401	3	374	66	96·1% (88·1 to 99·2)
6·4 years	401	4	372	70	95·3% (87·4 to 98·7)
≥ASC-US					
2·2 years	560	2	553	27	92·9% (70·0 to 98·3)
4·5 years	505	2	497	44	95·7% (83·5 to 99·5)
5·5 years	505	2	497	51	96·4% (86·3 to 99·6)
6·4 years	505	2	497	54	96·7% (87·3 to 99·6)
CIN1+					
2·2 years	560	0	553	6	100% (37·0 to 100)*
4·5 years	481	0	470	8	100% (42·4 to 100)
5·5 years	481	0	470	11	100% (61·5 to 100)
6·4 years	481	0	470	15	100% (73·4 to 100)
CIN2+					
2·2 years	560	0	553	3	100% (–26·2 to 100)*
4·5 years	481	0	470	5	100% (–7·7 to 100)
5·5 years	481	0	470	7	100% (32·7 to 100)
6·4 years	481	0	470	9	100% (51·3 to 100)

The values shown represent the cumulative number of women reporting an endpoint event associated with HPV 16/18 and related vaccine efficacy up to the maximum follow-up time for the different analyses undertaken: 2·2 years corresponds to the final analysis of the initial study (up to 2·2 years),[21] 4·5 years corresponds to the interim analysis of the combined initial and follow-up studies (up to 4·5 years),[22] 5·5 years corresponds to the interim analysis of the combined initial and follow-up studies (up to 5·5 years), and 6·4 years corresponds to the final analysis of the combined initial and follow-up studies (up to 6·4 years). All infections, cytology results, and lesions are associated with infection with either HPV 16 or HPV 18, or both. Incident infection reported in the according-to-protocol efficacy cohort (women who met all eligibility criteria, complied with study procedures, and had data available for efficacy measures). Atypical squamous cells of undetermined significance (ASC-US) or greater and cervical intraepithelial neoplasia (CIN) reported in the total vaccinated cohort (women who received at least one dose of study vaccine or placebo and for whom endpoint measures were available; this cohort was previously described[21,22] as an intention-to-treat cohort). CIN1+=CIN grade 1 and above. CIN2+= CIN grade 2 and above. *Calculation of vaccine efficacy against CIN at 2·2 years was a post-hoc evaluation.

Table 2: **Cumulative number of endpoint events associated with HPV 16/18**

	HPV-16/18 AS04-adjuvanted vaccine		Placebo		Vaccine efficacy (%; 95% CI)
	Total number of women	Women reporting ≥1 event	Total number of women	Women reporting ≥1 event	
≥ASC-US	505	118	497	162	35·4% (17·6–49·5)
≥LSIL	505	62	497	93	39·4% (15·6–56·8)
CIN1+	505	20	497	38	50·3% (12·5–72·6)
CIN2+	505	5	497	17	71·9% (20·6–91·9)

Combined analysis of the initial and follow-up studies in total vaccinated cohort (women who received at least one dose of study vaccine or placebo and for whom endpoint measures were available; this cohort was previously described[21,22] as an intention-to-treat cohort). ASC-US=atypical squamous cells of undetermined significance or greater. LSIL=low-grade squamous intraepithelial lesions or greater. CIN1+=cervical intraepithelial neoplasia grade 1 and above. CIN2+=cervical intraepithelial neoplasia grade 2 and above.

Table 3: **Vaccine efficacy against cytological and histopathological endpoints independent of HPV DNA in lesions up to 6·4 years of follow-up**

group (p=0·003 [log-rank test, descriptive analysis]; figure 2); vaccine efficacy was 59·8% (95% CI 20·5–80·7) during 6·4 years. Similarly, five women who had received the vaccine had incident infection with HPV 45 compared with 21 who had received placebo (p=0·0009 [log-rank test, descriptive analysis]; figure 2), corresponding to a vaccine efficacy of 77·7% (39·3–93·4). Few women had a 6-month persistent infection with HPV 31 (five in the vaccine group *vs* seven in placebo group) or HPV 45 (two *vs* three).

We recorded high vaccine efficacy against any cytological abnormal change of ASC-US or greater, or LSIL or greater, associated with HPV 16 or HPV 18, or both (table 1). A vaccine efficacy of 100% for both CIN1+ and CIN2+ associated with HPV 16 or HPV 18, or both, was achieved, with no vaccine recipients having a CIN event during 6·4 years. Table 2 summarises cumulative vaccine efficacy against incident infection with HPV 16 or HPV 18, or both, and against ASC-US or greater and CIN associated with HPV 16 or HPV 18, or both, for the initial study (up

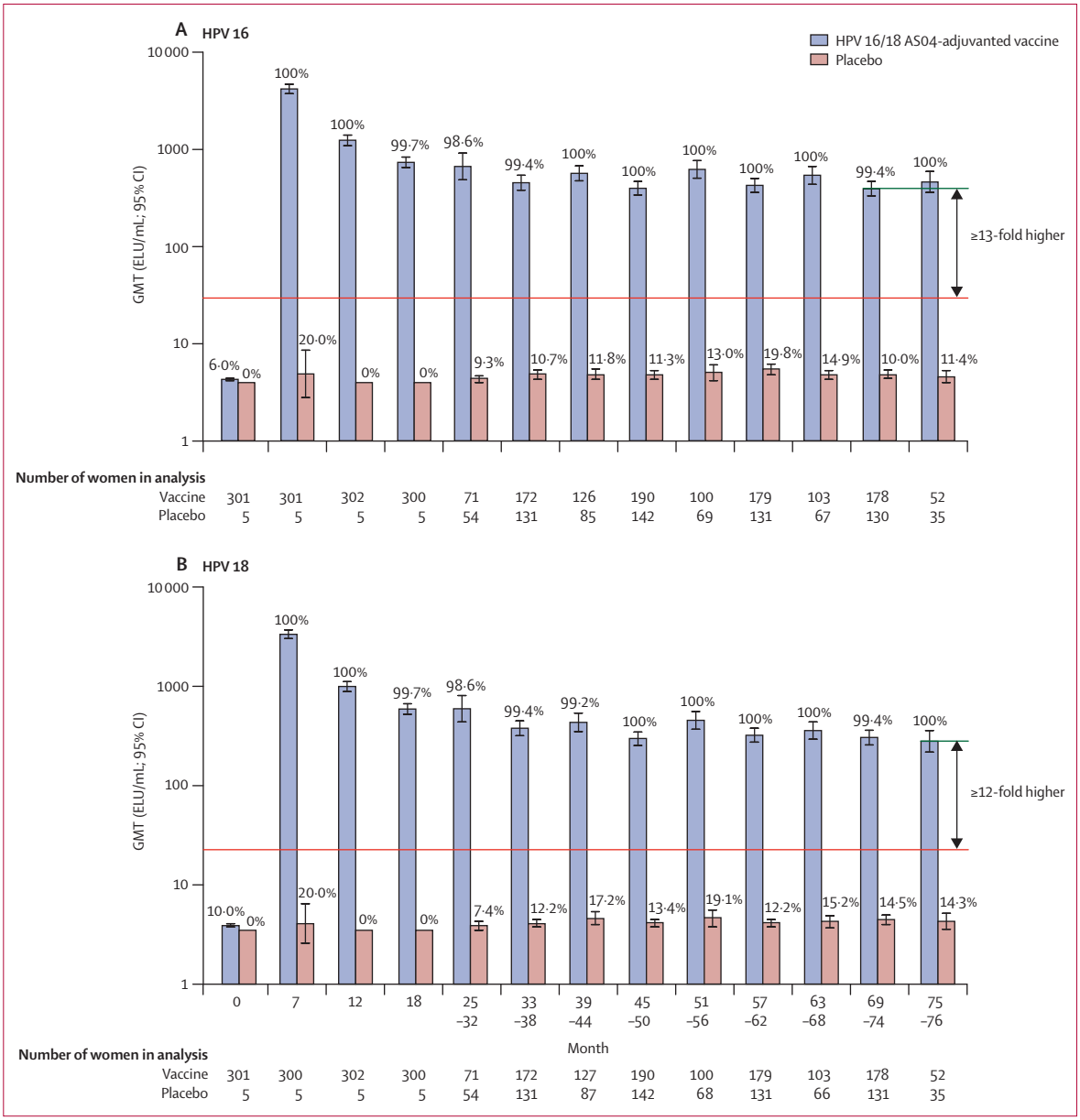

Figure 3: **Geometric mean titres for anti-HPV-16 (A) and anti-HPV-18 (B) IgG antibodies (ELISA)**
Combined analysis of initial and follow-up studies in according-to-protocol immunogenicity cohort. Red line represents the IgG antibody concentration assessed by ELISA in women from a phase 3 efficacy study who had cleared a natural infection.[20] Fold-difference is the difference between the natural infection concentration (red line) and the lowest IgG antibody concentration recorded during the final three intervals (months 63–68, 69–74, and 75–76; green line). Seropositivity (%) shown above bars. ELU/mL=ELISA units per mL.

to 2·2 years) and for the interim and final combined analyses of the initial and follow-up studies (up to 4·5, 5·5, and 6·4 years). With every year of follow-up, new cases of CIN2+ were identified, all of which were in the placebo group. These data show that vaccine efficacy against precancerous cervical lesions was sustained over time with continuous exposure to infection.

The analysis of cytohistological endpoints independent of HPV DNA in the lesion showed that vaccine efficacy against any cytological abnormal changes of ASC-US or greater and LSIL or greater was 35·4% (95% CI 17·6–49·5) and 39·4% (15·6–56·8), respectively. Vaccine efficacy against any CIN1+ and CIN2+ was also high (table 3). Few women in either the vaccine or placebo groups developed cytological endpoints or CIN lesions associated with HPV 31 and HPV 45 (data not shown).

Almost all women (99%) remained seropositive for anti-HPV-16 and anti-HPV-18 total IgG antibodies

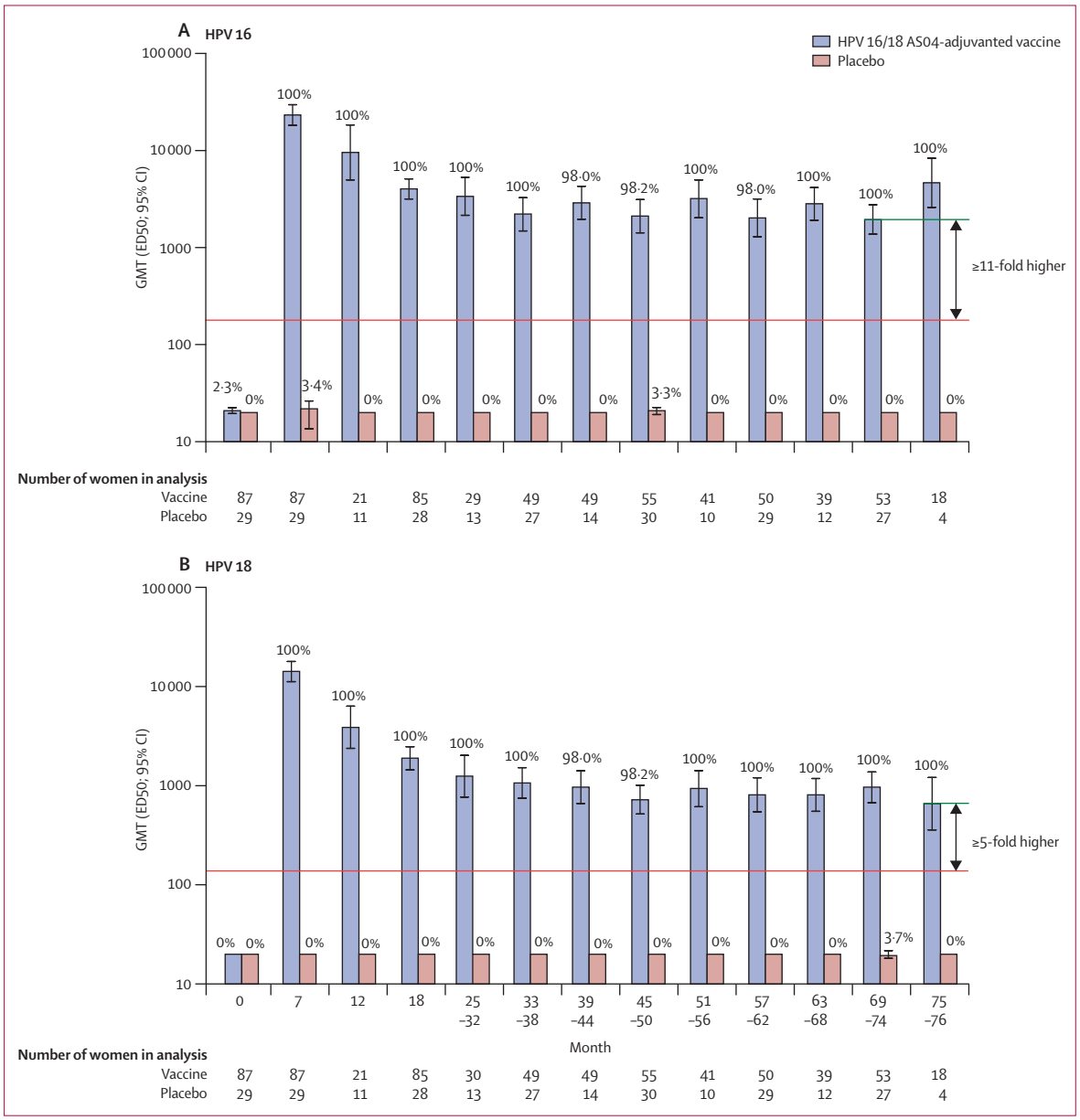

Figure 4: **Geometric mean titres for anti-HPV-16 and anti-HPV-18 neutralising antibodies (pseudovirion-based neutralisation assay)**
Combined analysis of initial and follow-up studies in subset of according-to-protocol immunogenicity cohort. Red line represents the neutralising antibody concentration assessed by pseudovirion-based neutralisation assay in women from a phase 3b study who had cleared a natural infection.[26] Fold-difference is the difference between the natural infection concentration (red line) and the lowest neutralising antibody concentration recorded during the final three intervals (months 63–68, 69–74, and 75–76; green line). Seropositivity (%) shown above bars. ED50=serum dilution causing a 50% reduction in secreted alkaline phosphatase activity compared with a control without serum.

	HPV-16/18 AS04-adjuvanted vaccine		Placebo	
	Number of events	Women reporting an event (%)	Number of events	Women reporting an event (%)
ATP safety cohort (N=373 in vaccine group and N=369 in placebo group)				
AEs	141	106 (28%)	199	123 (33%)
SAEs	35	30 (8%)	44	37 (10%)
NOCD	21	18 (5%)	23	21 (6%)
TVC (N=393 in vaccine group and N=383 in placebo group)				
AEs	149	110 (28%)	202	126 (33%)
SAEs	36	31 (8%)	46	39 (10%)
NOCD	21	18 (5%)	23	21 (5%)

According-to-protocol (ATP) safety cohort includes all evaluable women who did not use any investigational or non-registered product or any HPV vaccine other than study vaccine during the study period. Total vaccinated cohort (TVC) includes women who received at least one dose of study vaccine or placebo and for whom safety data were available. The protocol specified that medically significant disorders (ie, prompting emergency room or physician visits that were not related to common diseases) were to be recorded. However, an analysis of all adverse events (AEs) recorded was done. SAE=serious adverse event. NOCD=new onset chronic disease.

Table 4: **Occurrence of adverse events, serious adverse events, and new onset chronic disease in the follow-up study**

(figure 3). After a peak response at month 7, GMTs for both antibodies reached a plateau between months 18 and 24 after vaccination, thereafter remaining stable. During months 63–76 (the final three intervals during which antibody concentrations were recorded), antibody concentrations against HPV 16 and HPV 18 were at least 13-fold and at least 12-fold higher, respectively, than were concentrations recorded after clearance of a natural infection in a previous study.[20] Immunogenicity measured with the pseudovirion-based neutralisation assay in 162 participants showed a high level of seropositivity for functional antibodies (≥98%) and a similar time-course to the ELISA with respect to GMTs for both anti-HPV-16 and anti-HPV-18 neutralising antibodies (figure 4). Neutralising antibody concentrations were also substantially higher than were those recorded after clearance of a natural infection in a previous study.[26]

The safety profiles of the HPV-16/18 vaccine and placebo were similar (table 4). A similar number of women in both the vaccine and placebo groups reported adverse events. None of the serious adverse events was judged related or possibly related to vaccination, and there were no deaths. We recorded 130 pregnancies in the vaccine group and 131 in the placebo group, with no imbalance in outcomes between groups (data not shown).

Discussion

Findings from this study have shown that the HPV-16/18 AS04-adjuvanted vaccine in healthy women aged 15–25 years provides high, sustained efficacy up to 6·4 years against HPV-16/18 infection and cytohistological endpoints, associated with high and persistent concentrations of total and neutralising antibodies against HPV 16 and HPV 18. We also recorded cross-protection against HPV-31 and HPV-45 incident infection, and a favourable safety profile.

With consideration of all data from the follow-up period, we have shown 100% vaccine efficacy against CIN1+ and CIN2+ associated with HPV 16 or HPV 18, or both. The study population was continuously exposed to HPV infections, as indicated by the continuous accrual of CIN cases in the placebo group, showing that the vaccine confers sustained protection and that efficacy does not wane up to 6·4 years after first vaccination. Vaccine efficacy against persistent infection with HPV 16/18 remained 100%. HPV infection is increasingly being recognised as a potential surrogate indicator of risk for CIN grades 2–3 and high-grade squamous intraepithelial lesions, and thus of prophylactic vaccine efficacy.[27] Vaccine efficacy was very high against cytological ACS-US or greater associated with HPV 16/18. This finding is important because when such abnormal changes are identified, usual clinical practice dictates that women are referred for colposcopy, leading to anxiety and costs to health-care systems. Reduction of such follow-up and attendant treatment procedures would represent a vaccine benefit in addition to cervical cancer prevention.

Importantly, we have also shown high vaccine efficacy against cytohistological endpoints independent of HPV DNA. These data represent the overall amount of protection against HPV-related disease and thus are an indicator of the expected amount of overall vaccine benefit in the population. Although HPV 16/18 cause roughly 50% of CIN grades 2 and 3 lesions (high-grade squamous intraepithelial lesions),[6] vaccine efficacy in our study reached 71·9% against any CIN2+. This finding might be an indirect indication that the vaccine is able to confer some protection beyond HPV 16/18.

In support of this hypothesis, vaccine efficacy was achieved against incident infection with HPV 31 and HPV 45, two oncogenic types phylogenetically related to HPV 16 and HPV 18, respectively. HPV 31 and HPV 45 are among the types most frequently associated with cervical cancer after HPV 16 and HPV 18, and are responsible for 10% of all cervical cancer cases.[28] Furthermore, HPV 45 has been identified in as many as 10% of HPV-positive adenocarcinomas,[29] although HPV 31 is under-represented in this histological category.[7] The proportion of adenocarcinoma relative to all cervical cancer has increased in recent years, suggesting that screening practices are not as efficient at detecting it compared with squamous-cell carcinoma.[30] Few persistent infections and cytohistological endpoints associated with HPV 31 and HPV 45 were identified in either the vaccine or the placebo group, indicating their lower prevalence compared with HPV 16 and HPV 18. In a much larger efficacy study of the HPV-16/18 vaccine, significant vaccine efficacy was shown against CIN2+ associated with HPV 31 and HPV 45 in the TVC.[31]

Adolescents before sexual debut are the main target for immunisation because they are most likely to benefit from population-based HPV vaccination programmes. A strength of our study is that it was done in women who were naive to oncogenic HPV infection at the time of vaccination, and thus it attempts to represent the target population. Because the incidence of cervical cancer peaks on average more than 30 years after adolescence, the vaccine has to confer protection for many years. So far, our study shows the longest duration of protection against HPV 16 and HPV 18 infections for a licensed prophylactic HPV vaccine. Protection with the HPV-6/11/16/18 vaccine has been shown for up to 5 years after vaccination.[32]

The longlasting protection recorded in our study was associated with high and sustained immunogenicity of the vaccine. Concentrations of IgG antibodies against both antigens reached a plateau at least 12-fold higher than did those in women who had cleared a naturally acquired infection,[20] and were maintained throughout the follow-up period. These antibody kinetics correlate with sustained production, and are likely to indicate both the generation of long-lived plasma cells and the induction of memory B cells that replenish the plasma cell pool. The AS04-adjuvant system in the vaccine formulation is likely to be a key factor in its sustained immunogenicity. The vaccine produces consistently higher antibody titres that are sustained over a longer period and a more robust memory B-cell response than does a formulation containing aluminium salts alone as adjuvant.[17] Experience with other vaccines has suggested that the scale of the humoral response, together with memory B-cell induction, are both important for long-term protection.[33] On the basis of data generated in our study, a mathematical model has predicted that antibody concentrations will remain several-fold higher than will those associated with natural infection for at least 20 years for both antigens.[34]

Importantly, we showed the quality of the immune response, since a similar kinetic pattern for neutralising antibodies measured by the pseudovirion-based neutralisation assay was also detected for both HPV types. This assay[25] directly measures functional antibodies that are most relevant to vaccine studies, because neutralising antibodies are likely to be a major basis of protection against HPV infection.[9] Kemp and colleagues[35] showed a good correlation between results obtained with the pseudovirion-based neutralisation assay and GlaxoSmithKline's IgG ELISA assay, and between antibody concentrations elicited by the HPV-16/18 vaccine in serum and cervicovaginal secretions for both IgG and neutralising antibodies. This finding accords with a previous study suggesting that antibodies transude to the cervical epithelium.[36] So far, trials of HPV vaccine efficacy have not identified a correlate of protection.[8,37] However, neutralising antibodies are thought to mediate protection against HPV, and therefore, that the HPV-16/18 vaccine induces sustained high neutralising antibody concentrations considerably greater than those achieved after a natural infection[26] for both antigens is reassuring.

As seen in previous analyses of this study,[21,22] the vaccine is well tolerated. Few serious adverse events were reported, and pregnancy outcomes were similar between groups. In a pooled analysis of data from almost 30 000 girls and women participating in 11 studies (including our study), the vaccine was shown to be generally well tolerated, with a favourable safety profile in women of all ages.[23]

Loss of participants to follow-up is always a concern in long-term studies. Nevertheless, a substantial number of women enrolled into the follow-up study, and a large majority completed the study. Demographic characteristics at enrolment in the initial study were similar for women who entered the initial study and for those who enrolled in the follow-up study. Additionally, about the same number of women in both study groups continued into the follow-up study. These observations, together with the fact that the results of the follow-up study accorded with those of the initial study, suggest that participant attrition did not introduce a bias into the analysis.

The assessment of long-term efficacy is a crucial element in the evaluation of any HPV vaccine. To assess continuing protection, women in the placebo group have remained unvaccinated with the HPV-16/18 vaccine, despite the fact that potential benefits of vaccination are now clearly shown. We believe that this approach is justified because women in the placebo group had continuous access to cytological assessments and gynaecological care during the trial. No licensed vaccine was available when women were enrolled in the initial study, or when they were enrolled in the follow-up study. However, study participants were informed when any HPV vaccine was licensed in their country during the course of the study, and were given the option of withdrawing from the study at any time to receive it. The continued participation of participants was therefore based on full informed consent. A subset of women from our study is enrolled in a separate follow-up study continuing for up to 9·5 years after vaccination. Most women continuing in the separate study are now above the maximum age at which an HPV vaccine is approved for use, and would therefore not be able to receive any licensed vaccine under normal circumstances. All women provided informed consent to participate in these long-term follow-up studies.

In conclusion, the HPV-16/18 AS04-adjuvanted vaccine provides a high level of protection against HPV-16 and HPV-18 infection and associated cytohistological endpoints for up to 6·4 years. Additionally, it provides protection against cytohistological endpoints independent of HPV DNA and cross-protection against incident infection with HPV 31 and HPV 45. Efficacy is associated with high

and sustained concentrations of total and neutralising antibodies against HPV 16 and HPV 18. Although further assessment is necessary to confirm long-term vaccine effects, in view of the data from our study, we expect protection to continue for many more years.

Contributors

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Conflicts of interest

G Dubin, A Schuind, and T Zahaf are employees of GlaxoSmithKline Biologicals. All investigators at study clinical sites were funded through their institutions to undertake the study protocol. Additionally, F Aoki, S Barbier, M M Blatter, C Chambers, N S De Carvalho, S A Gall (Merck), D M Harper (Merck), J A Hedrick, D C Henry, R Kroll, A-B Moscicki, P S Naud, B Romanowski, W D Rosenfeld, R Somani, J C Teixeira, S K Tyring, C M Wheeler (Roche Molecular Systems and Merck), and D Ferris (Merck) were funded through their institutions to undertake other studies for GlaxoSmithKline and other companies as indicated. F Aoki, M M Blatter (Sanofi), C Chambers, N S de Carvalho, S A Gall (Merck), F A Guerra, D M Harper (Merck), P S Naud, B Romanowski, C M Roteli-Martins, J C Teixeira, C S Thoming, C M Wheeler, and A-B Moscicki (Merck) have participated in advisory boards, undertaken lectures, or provided consultancy for GlaxoSmithKline and other companies as indicated. D Ferris has participated in advisory board, provided consultancy, and undertaken lectures for Merck (not GSK). F Aoki is an investigator in GSK's HPV vaccine clinical trials but is not a member of any GSK HPV vaccine advisory board. He has participated in other advisory boards for GSK, undertaken lectures, and provided consultancies to GSK on antiviral drugs. W D Rosenfeld owns stock in GlaxoSmithKline Biologicals. S Barbier, P Colares de Borba, J A Hedrick, D C Henry, A P Korn, B Ramjattan, R M Shier, R Somani, and B J Sullivan declare that they have no conflicts of interest.

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