Sustained efficacy up to 4·5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial

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Summary

Background **Effective vaccination against HPV 16 and HPV 18 to prevent cervical cancer will require a high level of sustained protection against infection and precancerous lesions. Our aim was to assess the long-term efficacy, immunogenicity, and safety of a bivalent HPV-16/18 L1 virus-like particle AS04 vaccine against incident and persistent infection with HPV 16 and HPV 18 and their associated cytological and histological outcomes.**

Methods **We did a follow-up study of our multicentre, double-blind, randomised, placebo-controlled trial reported in 2004. We included women who originally received all three doses of bivalent HPV-16/18 virus-like particle AS04 vaccine (0·5 mL; n=393) or placebo (n=383). We assessed HPV DNA, using cervical samples, and did yearly cervical cytology assessments. We also studied the long-term immunogenicity and safety of the vaccine.**

Findings **More than 98% seropositivity was maintained for HPV-16/18 antibodies during the extended follow-up phase. We noted significant vaccine efficacy against HPV-16 and HPV-18 endpoints: incident infection, 96·9% (95% CI 81·3–99·9); persistent infection: 6 month definition, 94·3 (63·2–99·9); 12 month definition, 100% (33·6–100). In a combined analysis of the initial efficacy and extended follow-up studies, vaccine efficacy of 100% (42·4–100) against cervical intraepithelial neoplasia (CIN) lesions associated with vaccine types. We noted broad protection against cytohistological outcomes beyond that anticipated for HPV 16/18 and protection against incident infection with HPV 45 and HPV 31. The vaccine has a good long-term safety profile.**

Interpretation **Up to 4·5 years, the HPV-16/18 L1 virus-like particle AS04 vaccine is highly immunogenic and safe, and induces a high degree of protection against HPV-16/18 infection and associated cervical lesions. There is also evidence of cross protection.**

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Introduction

Cervical cancer is the second most common malignant disease in women worldwide, and generally affects individuals at a younger age than other cancers do.[1,2] Persistent infection with high-risk (oncogenic) human papillomavirus (HPV) genotypes is the main cause of cervical carcinogenesis.[3,4]

The association between HPV and cervical cancer is unique; no other major human cancer has a single necessary cause.[3,5] The relative risk of cervical cancer after infection with HPV, as indicated by the results of case-control studies, is the strongest causal relation in cancer epidemiology identified to date.[6,7] Establishment of the link between HPV and cervical cancer has provided the impetus for research into prophylactic vaccination against the most common HPV types associated with the disease—HPV 16 and HPV 18. Initial studies have provided evidence that L1 virus-like particle vaccines against HPV 16 and HPV 18 (as monovalent,[8] bivalent,[9] or quadrivalent[10] vaccines) prevent at least 90% of incident and persistent infections and their associated precursors of cervical cancer.

As predicted by mathematical modelling, the duration of protection provided by prophylactic HPV vaccination will be important in overall vaccine effectiveness.[11] Our aim, therefore, was to assess the long-term safety, immunogenicity, and efficacy of a bivalent HPV-16/18 L1 virus-like particle AS04 vaccine against incident and persistent infection with HPV 16 and HPV 18 and their associated cytological and histological outcomes.

Methods

Participants

Between November, 2003, and July, 2004, we enrolled women into the follow-up study of our double-blind, multicentre, randomised, placebo-controlled clinical trial,[9] assessing the safety, immunogenicity, and efficacy of a bivalent HPV-16/18 L1 virus-like particle AS04 vaccine against incident and persistent HPV-16/18 infections and their associated cytological and histological outcomes. Eligible women were those who participated in the initial efficacy study, received all three doses of vaccine or placebo, and for whom treatment allocation remained double blinded.

Written consent was mandatory for study participation and for study procedures, including colposcopy and loop electrosurgical excision. For girls younger than age 18 years, we obtained parental permission as well as their assent. The study was done at 28 sites in North America (Canada and the USA) and Brazil. The ethics committees of all sites approved the study protocol.

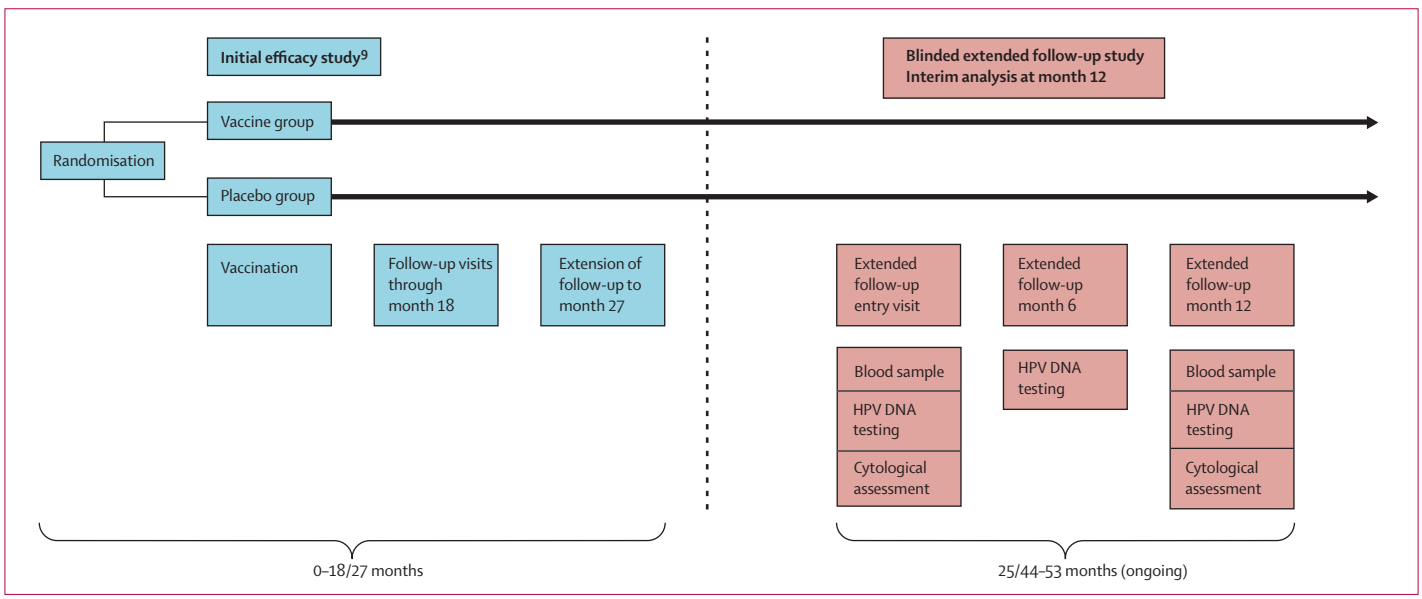

Figure 1: **Trial design**
Follow-up time for each woman in each study phase varies dependent on when she completed her last study visit in the initial efficacy study and when she entered the extended follow-up phase.

Procedures

Figure 1 shows the overall study design, including initial and follow-up phases. Here, we describe analyses done on data obtained at three study visits that occurred over about 1 year in the on-going double-blind follow-up study. No vaccines were administered in the extended follow-up phase.

In the initial efficacy study, we administered three doses (0, 1, 6 month schedule) of the bivalent HPV-16/18 virus-like particle AS04 vaccine (0·5 mL; GlaxoSmithKline Biologicals, Rixensart, Belgium), containing 20 µg each of HPV-16 and HPV-18 L1 virus-like particles produced on *Spodoptera frugiperda* Sf-9 and *Trichoplusia ni* Hi-5 cell substrate with AS04 adjuvant that contained 500 µg aluminum hydroxide and 50 µg 3-deacylated monophosphoryl lipid A per dose provided in a monodose vial. The placebo contained 500 µg of aluminium hydroxide per dose, and was identical in appearance to the vaccine. Blinding of treatment allocation was maintained for all participating women, investigators, study personnel, and personnel from GlaxoSmithKline who were directly involved with the undertaking of the study.

We did immunogenicity assays as previously described[9] with minor changes to the dilution series. Briefly, we detected antibodies with a type-specific ELISA, using type-specific recombinant virus-like particles as coating antigens. During the extended follow-up phase, we collected serum from participants at months 0 and 12 for assessment of immunogenicity. Seropositivity was defined as a titre greater than or equal to the assay threshold established at 8 units/mL (EU/mL) for HPV 16 and 7 EU/mL for HPV 18. We based time trends for seropositivity on geometric mean titres and corresponding 95% CIs. We identified the titres that resulted from natural infection by testing pre-vaccination blood samples obtained from women in the same countries as those in the initial study but who are participating in another ongoing HPV vaccine efficacy study; the sera assessed were from women who were seropositive for HPV 16 or HPV 18 and HPV DNA negative for the same HPV type.

We used cervical samples for HPV DNA testing, which we did every 6 months as previously described.[9] We assessed 14 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 11 low oncogenic risk HPV genotypes (6, 11, 34, 40, 42, 43, 44, 53, 54, 70, and 74), using the broad-spectrum PCR SPF_{10}-LiPA system (version 1 Labo Biomedical Products, Rijswijk, Netherlands). In addition, we used type-specific PCR methods for detection of HPV 16 and HPV 18 (all PCR analyses done at Delft Diagnostic Laboratory, Voorburg, Netherlands). We also used this PCR technology for HPV DNA testing in the biopsy samples. To reduce the risk of false-negative PCR results in the biopsy samples, the biopsy PCR testing algorithm included steps to prevent or remove inhibition, including microdissection of lesions, when appropriate, and dilution of samples.

Health-care providers collected cervical samples in PreservCyt medium (Cytyc Corporation, Boxborough, MA, USA) at 6-month intervals; no cervicovaginal samples were obtained in the follow-up phase of the study. A central laboratory (Quest Diagnostics, Teterboro, NJ, USA) assessed liquid-based cytological samples at month 0 and 12. We did cytology assessments at 6-month intervals if previous findings showed atypical squamous cells and positivity by Hybrid Capture II (HCII; Digene Corp, Gaithersburg, MD, USA). We report results with the 2001 Bethesda classification system.[12]

With respect to colposcopy and treatment referral, protocol guidelines recommended colposcopy after two consecutive or intermittent reports of atypical squamous cells of undetermined significance (if HCII high-risk HPV DNA positive) or low-grade squamous intraepithelial lesions (independent of HPV DNA results), or one report of atypical glandular cells, atypical squamous cells cannot exclude high-grade squamous intraepithelial lesions, or high-grade squamous intraepithelial lesions. Biopsy was also required for any suspected lesions on colposcopy. Finally, guidelines directed excisional treatment for unexplained atypical glandular cells, biopsy results for adenocarcinoma in situ, and cervical intraepithelial neoplasia (CIN)2 or worse.

We undertook histological diagnosis of formalin-fixed tissue specimens in two stages. A diagnosis for clinical management was made by at least two gynaecological histopathologists in a central laboratory. All diagnoses of CIN were confirmed by an independent re-examination of the tissue sections by a separate review panel of expert pathologists for endpoint determination. At least two members of the endpoint review panel identified and graded lesions by independent examination. If there was disagreement between diagnoses, a third expert pathologist examined the sections. The endpoint diagnoses were determined by a simple majority rule. We obtained digital images of the sections and marked the lesions to assist further dissection for PCR analyses. We sent this material to Delft Diagnostics together with tissue blocks for sectioning and microdissection followed by HPV DNA testing. Any suspected change in histological grade or identification of additional lesions within the additional sections required re-examination by the endpoint review panel. We graded histological diagnosis as follows: CIN1+ was defined as CIN1, 2, 3, adenocarcinoma in situ, and invasive carcinoma; CIN2+ included all listed CIN1+ categories, excluding CIN1. All study personnel remain blinded to HPV DNA test results, except HCII positive results, and were only informed of cytological and histological diagnoses for clinical management purposes.

We defined incident cervical infection as the first detection of HPV 16, HPV 18, or other important high-risk types (45, 31, 33, 52, and 58) in the liquid-based cytological sample. Persistent infection with HPV 16 or HPV 18 required at least one detection of the relevant HPV type in the cervical sample from the extended follow-up study. A 6-month definition required the detection of the same HPV type in two consecutive assessments, with no negative sample in between, over a minimum of 5 months; a 12-month definition required the detection of the same HPV type at consecutive assessments, with no negative samples in between, over a minimum of 10 months.

Women reported as adverse events any new onset of chronic disease defined according to guidelines of the *Medical Dictionary for Regulatory Activities* (MedDRA); conditions that prompted a visit to a doctor or an emergency room and not related to common diseases; and serious adverse events that occurred between the last visit of the initial efficacy study and during the extended follow-up phase. Blinding continued for treatment allocation unless deemed essential for management of any safety event reported.

Our primary aim was to investigate long-term vaccine efficacy in the prevention of incident HPV-16/18 infection in young women who participated in our initial efficacy study.[9] Our secondary objectives were to assess long-term vaccine efficacy in the prevention of HPV-16/18 infections,

	Month 25–32* (n [n_i])	Month 33–38* (n [n_i])	Month 39–44* (n [n_i])	Month 45–50* (n [n_i])	Month 51–53* (n [n_i])
Vaccine group					
Visit 1 (n=393)	91 (91)	217 (217)	85 (85)	0	0
Visit 2 (n=377)	8 (0)	84 (0)	221 (0)	64 (0)	0
Visit 3 (n=367)	0	3 (3)	78 (77)	232 (230)	54 (53)
Placebo group					
Visit 1 (n=383)	98 (98)	212 (212)	73 (73)	0	0
Visit 2 (n=371)	6 (0)	81 (0)	226 (0)	58 (0)	0
Visit 3 (n=365)	0	3 (3)	80 (80)	232 (231)	50 (50)

*Total follow-up from start of initial efficacy study to month 12 extended follow-up. n (n_i)=number of women who attended visit, according to month-interval (number of women who attended visit where evaluable blood sample collected).

Table 1: **Number of women who completed visits in extended follow-up phase, according to timing of visit relative to their enrolment in initial efficacy study**

	Extended follow-up phase		Combined initial efficacy study and extended follow-up phase	
	Vaccine	Placebo	Vaccine	Placebo
Intention-to-treat analysis	393	383	560	553
According-to-protocol analysis				
Immunogenicity	310	249	384	344
Safety	373	371	540	541
Efficacy	350	344	473	470

Numbers represent maximum potential eligible women for all analyses. However, some women might have been censored because of endpoint occurrence.

Table 2: **Numbers of women, according to type of analysis**

	Initial efficacy study		Extended follow-up phase	
	Vaccine (n=560)	Placebo (n=553)	Vaccine (n=393)	Placebo (n=383)
Age (years), mean (SD)	20·4 (2·8)	20·5 (2·7)	23·2 (2·9)	23·2 (2·8)
Region				
North America*	302 (54%)	305 (55%)	163 (41%)	165 (43%)
Brazil	258 (46%)	248 (45%)	230 (59%)	218 (57%)
Ethnic origin				
White	389 (69%)	384 (69%)	251 (64%)	254 (66%)
Black	43 (8%)	41 (7%)	33 (8%)	29 (8%)
Asian	9 (2%)	4 (1%)	8 (2%)	5 (1%)
Other	119 (21%)	124 (22%)	101 (26%)	95 (25%)

Data are number (%) unless otherwise indicated. *Includes Canada and USA.

Table 3: **Characteristics of women included in intention-to-treat analyses**

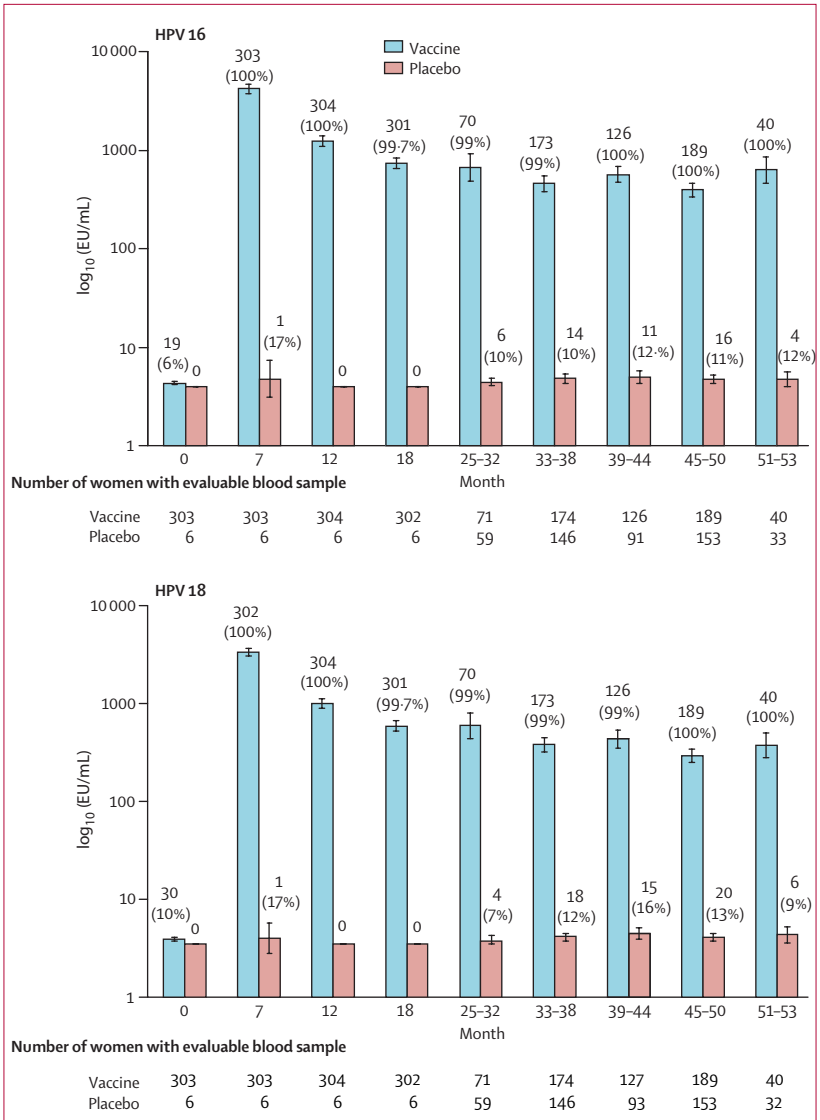

***Figure 2*: Geometric mean titres and seropositivity rates, according to HPV type and group in according-to-protocol analyses for immunogenicity**

%=proportion of women seropositive. Sera from all vaccinees and a small number of samples from the placebo group of the initial study were retested.

persistent for 6 months and 12 months; and cytological outcomes, including low-grade squamous intraepithelial lesions and high-grade squamous intraepithelial lesions, atypical squamous cells of undetermined significance, atypical squamous cells cannot exclude high-grade squamous intraepithelial lesions, and atypical glandular cells associated with HPV-16/18 infection. We also investigated vaccine efficacy against incident infection associated with other high-risk types of HPV, and long-term vaccine efficacy in preventing histopathological endpoints, including CIN associated with HPV 16 or HPV 18. We added prevention of cytohistological outcomes independent of HPV DNA type post-hoc to the analyses. Other objectives included the assessment of persistence of vaccine-induced immune responses and long-term safety of the vaccine.

Statistical analysis

We estimated that a minimum of 500 women from the initial study would be needed to achieve 80% power to confirm that the lower limit of the 95% CI of the vaccine efficacy estimate for incident infection was above 0 at the time of final study analysis, assuming a minimum of 70% vaccine efficacy.[13] An α of 0·001 (two-sided test) was allocated for this interim analysis[14] for efficacy, safety, and immunogenicity. This α level was defined on the basis of the expected number of analyses for the extended follow-up data exclusively. We also present estimates of vaccine efficacy for the combined data, including the initial and extended follow-up phases, with their associated p values. We did not use the latter for inferential purposes; they are given solely as further descriptors of the intervention effects that are best represented by the vaccine efficacy estimates and their respective 95% CIs.

We defined four sets of analyses: intention-to-treat analysis and according-to-protocol analysis for women in the extended follow-up phase, and intention-to-treat analysis and according-to-protocol analysis for the combined cohorts from the initial efficacy study and the extended follow-up phase. We censored women from assessment in the extended follow-up analyses if a defined endpoint associated with HPV 16/18 occurred in the initial efficacy study. We also censored women from type-specific assessment if an incident infection associated with any other high-risk HPV type had been detected in the initial efficacy study. In the combined analyses, follow-up time used to calculate vaccine efficacy in the intention-to-treat analyses started at first vaccination (month 0 of the initial efficacy study), and in the according-to-protocol analyses at the completion of vaccination (after 6 months) in the initial efficacy study. An independent external statistician did the interim analysis to maintain study blinding.

For the immunogenicity analysis, we included women with serology results who met study eligibility criteria and assessment criteria, and had at least one timepoint at which antibodies were detected for at least one vaccine antigen component. We excluded women from this analysis if HPV-16/18 infection had been detected at any point. We calculated 95% CI for seropositivity rates and geometric mean titres.

In the according-to-protocol analysis, we included all women in the extended follow-up phase who received three doses of HPV-16/18 L1 virus-like particle AS04 vaccine or placebo, and who were negative for high-risk HPV DNA and seronegative for HPV 16 and HPV 18 at month 0, and negative for HPV-16 and HPV-18 DNA at month 6 in the initial efficacy study. In the intention-to-treat analyses, we included all women who had received at least one dose of study vaccine or placebo in the initial efficacy study, and who had any data available for outcome measurement in the extended follow-up phase.

	Vaccine			Placebo			Vaccine efficacy, % (95% CI)	p
	Total women	Women reporting ≥1 HPV-16/18 event	Event rate (95% CI)*	Total women	Women reporting ≥1 HPV-16/18 event	Event rate (95% CI)*		
According-to-protocol analyses of extended follow-up phase (vaccine: n=350, placebo: n=344)								
HPV 16	311	1	0·2 (0·0–0·9)	280	21	4·0 (2·5–6·2)	95·8 (73·6–99·9)	<0·0001
HPV 18	310	0	0·0 (0·0–0·6)	291	11	2·0 (1·0–3·5)	100·0 (62·1–100)	0·0003
HPV 16/18	310	1	0·2 (0·0–0·9)	277	28	5·6 (3·7–8·1)	96·9 (81·3–99·9)	<0·0001
Intention-to-treat analyses of extended follow-up phase (vaccine: n=393, placebo: n=383)								
HPV 16	353	2	0·3 (0·0–1·1)	322	25	4·2 (2·7–6·2)	92·7 (70·9–99·2)	<0·0001
HPV 18	356	0	0·0 (0·0–0·5)	332	12	1·9 (1·0–3·3)	100·0 (65·6–100·0)	0·0001
HPV 16/18	352	2	0·3 (0·0–1·1)	313	31	5·5 (3·7–7·8)	94·4 (77·9–99·3)	<0·0001
According-to-protocol analyses of combined initial and follow-up phase (vaccine: n=473, placebo: n=470)								
HPV 16	414	1	0·1 (0·0–0·6)	385	40	4·5 (3·2–6·2)	97·7 (86·6–99·9)	<0·0001
HPV 18	414	2	0·2 (0·0–0·7)	385	17	1·9 (1·1–3·0)	88·9 (53·3–98·8)	0·0002
HPV 16/18	414	3	0·3 (0·1–0·9)	385	51	5·9 (4·4–7·7)	94·7 (83·5–98·9)	<0·0001
Intention-to-treat analyses of combined initial and follow-up phase (vaccine: n=560, placebo: n=553)								
HPV 16	481	7	0·5 (0·2–1·1)	470	55	4·4 (3·3–5·8)	88·0 (73·6–95·4)	<0·0001
HPV 18	481	3	0·2 (0·0–0·6)	470	29	2·3 (1·5–3·2)	90·0 (67·8–98·1)	<0·0001
HPV 16/18	481	9	0·7 (0·3–1·3)	470	73	6·0 (4·7–7·5)	88·5 (77·0–95·0)	<0·0001

*Per 100 person-years: number of cases divided by accrued person-time.

Table 4: **Vaccine efficacy for incident HPV-16/18 infections, in cervical samples**

For intention-to-treat analyses of efficacy against incident infection with each individual high-risk HPV type, other than HPV 16/18, we included women who had received at least one vaccine dose and were HPV DNA negative for the specific HPV type at month 0 in the initial study. The follow-up time for all efficacy analyses ended at the time of an outcome event or at the last visit for which data were available. We express event rates as the number of cases divided by the accrued person-time since enrolment into the initial study.

We used the conditional exact method to calculate vaccine efficacy for HPV-16/18 infection, and for other oncogenic HPV types. This method controls for differences in follow-up time between groups. We defined vaccine efficacy as one minus the ratio between the incidence rates in the vaccinated versus placebo groups, and the respective 95% CI descriptively measured the precision of the estimates.

We tabulated safety event variables by the number of women who reported a symptom and the number of symptoms reported. For the analyses according to protocol, we included all enrolled women who complied with specified, minimum protocol requirements; for the intention-to-treat analyses we included women who had any safety data available.

We did all analyses with SAS (version 8.2; SAS Institute, Cary, North Carolina, USA) and ProcStatXact 5 (Cytel, Cambridge, MA, USA).

Role of the funding source

This study was funded and coordinated by GlaxoSmithKline Biologicals. Main investigators and co-investigators in the HPV Vaccine Study group obtained data for the study and cared for the patients. GlaxoSmithKline Biologicals did all HPV serological testing, Quest Diagnostics processed all cytology and histology specimens, and Delft Diagnostic Laboratory undertook PCR for HPV types. To ensure study blinding, all statistical analyses were done by external independent statisticians. The findings presented are included in the study report prepared by the sponsor and used for regulatory purposes. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 776 women enrolled in the extended follow-up study (mean follow-up time 47·7 months, SD 3·4), 732 (94%) completed visit 3 (table 1). Table 2 shows the numbers of women included in the intention-to-treat and according-to-protocol analyses. Table 3 shows the characteristics of the participants; the average age of participants was 23 years (SD 3). We noted similar patterns of co-factors for HPV acquisition in women in the vaccine and placebo groups at entry into the extended follow-up phase of the study and at the last study visit (see webtable).

See **Online** for webtable

More than 98% of women in the vaccine group were seropositive for HPV 16 and HPV 18 at every timepoint (figure 2) during the extended follow-up phase of the study. We noted a decline in geometric mean titre values from peak responses 1 month after the third vaccine (month 7 of initial study) to a stable plateau beginning at month 18; overall there was a less than one $\log_{10}$ decline in geometric mean titre values from peak values to end of follow-up. There was at least a 133-fold difference in

	Definition of persistence (months)	Vaccine			Placebo			Vaccine efficacy, % (95% CI)	p
		Total women	Women reporting ≥1 HPV-16/18 event	Event rate (95% CI)*	Total women	Women reporting ≥1 HPV-16/18 event	Event rate (95% CI)*		
According-to-protocol analyses of extended follow-up phase (vaccine: n=350, placebo: n=344)									
HPV 16	6	311	1	0·2 (0·0 to 0·9)	287	12	2·2 (1·1 to 3·8)	92·3 (47·7 to 99·8)	0·0012
HPV 18	6	311	0	0·0 (0·0 to 0·6)	295	5	0·9 (0·3 to 2·0)	100·0 (–5·5 to 100·0)	0·0269
HPV 16/18	6	311	1	0·2 (0·0 to 0·9)	287	16	3·0 (1·7 to 4·8)	94·3 (63·2 to 99·9)	<0·0001
HPV 16	12	311	0	0·0 (0·0 to 0·6)	295	5	0·9 (0·3 to 2·0)	100·0 (–5·2 to 100·0)	0·0269
HPV 18	12	311	0	0·0 (0·0 to 0·6)	295	2	0·3 (0·0 to 1·2)	100·0 (–419·4 to 100·0)	0·2366
HPV 16/18	12	311	0	0·0 (0·0 to 0·6)	295	7	1·2 (0·5 to 2·5)	100·0 (33·6 to 100·0)	0·0062
Intention-to-treat analyses of extended follow-up phase (vaccine: n=393, placebo: n=383)									
HPV 16	6	357	1	0·1 (0·0 to 0·8)	331	16	2·6 (1·5 to 4·1)	94·2 (62·4 to 99·9)	<0·0001
HPV 18	6	358	0	0·0 (0·0 to 0·5)	342	6	0·9 (0·3 to 2·0)	100·0 (16·5 to 100·0)	0·0133
HPV 16/18	6	357	1	0·1 (0·0 to 0·8)	329	19	3·1 (1·9 to 4·8)	95·2 (69·6 to 99·9)	<0·0001
HPV 16	12	357	0	0·0 (0·0 to 0·5)	341	8	1·2 (0·5 to 2·4)	100·0 (43·0 to 100·0)	0·0031
HPV 18	12	358	0	0·0 (0·0 to 0·5)	344	3	0·4 (0·1 to 1·3)	100·0 (–141 to 100·0)	0·1171
HPV 16/18	12	357	0	0·0 (0·0 to 0·5)	340	10	1·5 (0·7 to 2·8)	100·0 (57·0 to 100·0)	0·0007
According-to-protocol analyses of combined initial and follow-up phase (vaccine: n=473, placebo: n=470)									
HPV 16	6	414	1	0·1 (0·0 to 0·6)	385	19	2·1 (1·2 to 3·2)	95·1 (69·2 to 99·9)	<0·0001
HPV 18	6	414	0	0·0 (0·0 to 0·4)	385	5	0·5 (0·2 to 1·2)	100·0 (–3·5 to 100·0)	0·0256
HPV 16/18	6	414	1	0·1 (0·0 to 0·6)	385	23	2·5 (1·6 to 3·8)	96·0 (75·2 to 99·9)	<0·0001
HPV 16	12	414	0	0·0 (0·0 to 0·4)	385	7	0·7 (0·3 to 1·5)	100·0 (34·3 to 100·0)	0·0059
HPV 18	12	414	0	0·0 (0·0 to 0·4)	385	2	0·2 (0·0 to 0·8)	100·0 (–408·2 to 100·0)	0·2319
HPV 16/18	12	414	0	0·0 (0·0 to 0·4)	385	9	1·0 (0·4 to 1·8)	100·0 (52·2 to 100·0)	0·0013
Intention-to-treat analyses of combined initial and follow-up phase (vaccine: n=560, placebo: n=553)									
HPV 16	6	481	2	0·1 (0·0 to 0·5)	470	29	2·3 (1·5 to 3·2)	93·4 (74·0 to 99·2)	<0·0001
HPV 18	6	481	0	0·0 (0·0 to 0·3)	470	8	0·6 (0·3 to 1·2)	100·0 (42·8 to 100·0)	0·0035
HPV 16/18	6	481	2	0·1 (0·0 to 0·5)	470	34	2·7 (1·8 to 3·7)	94·4 (78·2 to 99·4)	<0·0001
HPV 16	12	481	1	0·1 (0·0 to 0·4)	470	13	1·0 (0·5 to 1·7)	92·5 (50·3 to 99·8)	0·0008
HPV 18	12	481	0	0·0 (0·0 to 0·3)	470	3	0·2 (0·0 to 0·7)	100·0 (–138·0 to 100·0)	0·1203
HPV 16/18	12	481	1	0·1 (0·0 to 0·4)	470	16	1·2 (0·7 to 2·0)	94·0 (61·1 to 99·9)	0·0001

*Per 100 person-years: number of cases divided by accrued person–time.

Table 5: **Vaccine efficacy for persistent HPV-16/18 infections, in cervical samples**

geometric mean titre values between the vaccine and placebo groups for both HPV 16 and HPV 18 at the end of the extended follow-up period. The geometric mean titre values associated with naturally-acquired HPV-16 infection were 36·3 EU/mL (95% CI 33·8–38·9) and for HPV-18 26·5 (24·5–28·8). Vaccine-induced geometric mean titres at 51–53 months were about 17-fold and 14-fold higher in HPV-16 and HPV-18 infections, respectively, than noted for natural infection.

We noted significant long-term vaccine efficacy against incident HPV-16/18 infections in all of the extended follow-up analyses a mean of 42 months after completion of the vaccination schedule (table 4). Likewise, in the combined initial and extended follow-up study analyses, we noted high levels of vaccine efficacy through a mean follow-up period of 47·7 months after entry to initial study.

We noted a high level of vaccine efficacy with both the 6-month and 12-month definitions of persistent HPV-16 and HPV-18 infections in the follow-up study and in analyses of the two study phases combined, although not all findings were significant because of the limited number of events (table 5). One woman in the vaccine group had a 6-month persistent HPV-16 infection detected at the last two timepoints of the follow-up study. Vaccine efficacy against 12-month persistent infection with HPV 16/18 was 100% in the combined initial and follow-up analysis that was done per-protocol. We also noted substantial vaccine efficacy in preventing 6-month and 12-month persistent HPV-18 infection irrespective of the type of analyses.

As shown in table 6, the HPV-16/18 L1 virus-like particle AS04 vaccine was highly efficacious against cytological abnormalities (atypical squamous cells of undetermined significance or worse and low-grade squamous intraepithelial lesions or worse) associated with HPV 16/18, in the combined analyses of initial and follow-up phases. We continue to note 100% vaccine efficacy against all histological abnormalities associated with HPV 16/18. We detected no lesions related to HPV 18. Table 6 also shows substantial vaccine efficacy against abnormal

	Endpoint	Vaccine		Placebo		Vaccine efficacy, % (95% CI)	p
		Total women	Women reporting an event	Total women	Women reporting an event		
HPV 16	≥ASCUS	505	1	497	32	97·0 (82·2 to 99·9)	<0·0001
	≥LSIL	505	1	497	22	95·6 (73·0 to 99·9)	<0·0001
	CIN1+	481	0	470	8	100·0 (42·4 to 100·0)	0·0035
	CIN2+	481	0	470	5	100·0 (–7·7 to 100·0)	0·0292
HPV 18	≥ASCUS	505	1	497	17	94·3 (63·8 to 99·9)	<0·0001
	≥LSIL	505	1	497	6	83·8 (–33·7 to 99·6)	0·0674
	CIN1+	481	0	470	0	NA	NA
	CIN2+	481	0	470	0	NA	NA
HPV 16/18	≥ASCUS	505	2	497	44	95·7 (83·5 to 99·5)	<0·0001
	≥LSIL	505	2	497	26	92·6 (70·5 to 99·2)	<0·0001
	CIN1+	481	0	470	8	100·0 (42·4 to 100·0)	0·0035
	CIN2+	481	0	470	5	100·0 (–7·7 to 100·0)	0·0292
Any high-risk HPV type*	≥ASCUS	505	53	497	95	48·4 (27·0 to 63·8)	0·0001
	≥LSIL	505	30	497	61	53·4 (26·7 to 71·0)	0·0006
	CIN1+	481	8	470	19	58·7 (1·3 to 84·4)	0·0315
	CIN2+	481	3	470	9	67·1 (–31·9 to 94·3)	0·0869
Independent of HPV DNA status†	≥ASCUS	505	90	497	138	39·8 (20·9 to 54·4)	0·0002
	≥LSIL	505	41	497	70	44·6 (17·4 to 63·3)	0·0034
	CIN1+	505	12	497	24	51·5 (–0·9 to 77·9)	0·0418
	CIN2+	505	3	497	11	73·3 (–1·0 to 95·2)	0·0327

ASCUS (atypical squamous cells of undetermined significance) and LSIL (low-grade squamous intraepithelial lesions) refer to cytological outcomes; CIN refers to histological outcomes based on cervicovaginal (initial study) and cervical samples (initial study and extended follow-up) combined. CIN1+ is defined as CIN1, 2, 3, and invasive cell carcinoma (ICC), and CIN2+ is CIN2, CIN3, and ICC. *Includes HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Total number of events include multiple type infections, which may or may not include HPV 16 or HPV 18 single or combined infections. †Includes outcomes also associated with low-risk types and HPV DNA negative.

Table 6: **Overview of combined initial and extended follow-up vaccine efficacy against cytological and histological endpoints**

	Vaccine			Placebo			Vaccine efficacy (%) (95% CI)
	Total women	Women reporting ≥1 event of HPV 45, HPV 31, HPV 33, HPV 52, or HPV 58 and who did not report the same event in initial study	Event rate (95% CI)*	Total women	Women reporting ≥1 event of HPV 45, HPV 31, HPV 33, HPV 52, or HPV 58 and who did not report the same event in initial study	Event rate (95% CI)*	
HPV 45	528	1	0·1 (0·0 to 0·4)	518	17	1·2 (0·7 to 1·9)	94·2 (63·3 to 99·9)
HPV 31	528	14	0·9 (0·5 to 1·6)	516	30	2·1 (1·4 to 3·0)	54·5 (11·5 to 77·7)
HPV 33	529	12	0·8 (0·4 to 1·4)	519	13	0·9 (0·5 to 1·5)	8·6 (–117·3 to 61·9)
HPV 52	524	40	2·8 (2·0 to 3·8)	515	48	3·5 (2·6 to 4·6)	18·6 (–26·5 to 47·8)
HPV 58	529	14	0·9 (0·5 to 1·6)	517	16	1·1 (0·6 to 1·8)	14·0 (–87·9 to 61·1)

*Per 100 person-years: number of cases divided by accrued person–time.

Table 7: **Vaccine efficacy against incident infection with HPV 45, HPV 31, HPV 52, HPV 33, and HPV 58 in cervical samples from intention-to-treat analyses**

cytological and histological outcomes associated with any high-risk HPV type and independently of HPV DNA status. Table 7 shows substantial vaccine efficacy against incident infection with HPV 45 and HPV 31.

More women in the placebo group than in the vaccine group reported adverse events and new onset of chronic diseases (table 8). A comparable number of women in both vaccine and placebo groups reported serious adverse events; none was judged related, or possibly related, to vaccination. No one died.

Discussion

Our findings indicate that the HPV-16/18 L1 virus-like particle AS04 vaccine has sustained long-term vaccine efficacy against incident and persistent infections associated with HPV 16 and HPV 18. The results show sustained immune response and long-term efficacy against HPV-16 and HPV-18 infection, including persistence up to 12 months, and against related cytohistological outcomes as well as providing evidence of broader protection against cytohistological outcomes and cross-protection against HPV 45 and HPV 31. The vaccine has a good safety record.

The AS04 adjuvant system used to formulate the vaccine could be contributing to the maintenance of the sustained immune response. Clinical trials of other vaccines also show higher antibody titres when adjuvanted with AS04 than with aluminum alone.[15,16]

	Vaccine (n=373)	Placebo (n=371)
Unsolicited adverse event		
Number of women with at least one adverse event reported	54 (14%)	81 (22%)
Number of adverse events reported	65	98
NOCD		
Number of women with at least one NOCD event reported	10 (3%)	18 (5%)
Number of NOCD events reported	10	19
Serious adverse events		
Number of women with at least one serious adverse event reported	16 (4%)	19 (5%)
Number of serious adverse events reported	21	19

Analyses according to protocol. Categories of new onset chronic disease (NOCD) include (not necessarily occurring) immune system disorders: endocrine, musculoskeletal, and connective tissue, metabolism and nutrition, respiratory and thoracic disorder.

Table 8: **Reported adverse events**

We noted a high degree of protection against both incident and persistent infections of at least 6 months and 12 months duration up to 4·5 years of follow-up in the combined assessment of the initial and extended follow-up studies. The high degree of protection lends support to the notion that persistent HPV infection is a valid virological endpoint in the clinical assessment of HPV vaccines.[17] Given that persistent HPV infection is a valid intermediate endpoint for the development of high-grade dysplasia and cervical cancer, the high level of efficacy seen here against persistent infection might ultimately lead to the long-term prevention of HPV-16 and HPV-18 associated precancerous and cancerous lesions.

It is noteworthy that persistent HPV-16 infection was consistently detected in the cytology samples preceding all instances of CIN detected. The continued use of the SPF_{10}-LiPA system followed by type-specific PCR allowed for consistency of HPV detection between cytological and histological specimens, reducing the possibility of misclassification of HPV status.

Our clinical management algorithm allowed for a sufficient amount of time before tissue sampling to improve the distinction between naturally regressing lesions and the progressing clinically significant HPV-16 and HPV-18 precancerous lesions. This algorithm required colposcopy referral after two atypical squamous cells of undefined significance (if high-risk HPV positive) or two cytology reports of low-grade squamous intraepithelial lesions, which probably resulted in detection of a high proportion of clinically important and persistent cervical lesions and avoided the overdiagnosis and overtreatment of frequently regressing CIN1 and CIN2 lesions.[18] The few observations of CIN in the extended follow-up phase might reflect the time it takes to develop true persistent high-grade cervical dysplasia.[19] The main limitation of this study is the few observations of CIN in the extended follow-up phase, which might reflect the time it takes to develop true persistent high-grade cervical dysplasia.[17]

Reports on the safety of HPV vaccines have focused on standard safety variables, such as injection site and solicited and unsolicited and general and serious adverse events. Here, we present safety data for this bivalent HPV-16/18 L1 virus-like particle AS04 vaccine up to 53 months. Additionally, our observations of vaccine safety are consistent with the clinically acceptable safety profile of other AS04-based vaccines.[20] We noted that more women on placebo than women who received the vaccine reported at least one adverse event. One possible explanation for this finding is that the women who received the vaccine had fewer cytological abnormalities that required diagnostic follow-up; controls probably attended more colposcopy sessions, providing an increased opportunity to report adverse events.

When we estimated vaccine efficacy against cytological or histological endpoints associated with all high-risk HPV types and independent of HPV status, we noted protection that seems to extend beyond the degree of effect that might be explained simply by protection against HPV-16/18 endpoints alone. Analyses of vaccine efficacy against incident infection with other important oncogenic HPV types indicate a high degree of protection against HPV 45 and protection against HPV 31, the third and fourth most common HPV types associated with cervical cancers. Analyses of lesions associated with high-risk types other than HPV 16 and HPV 18 are confounded by a high frequency of multiple infections, however, including HPV 16 and HPV 18. Cross protection against other oncogenic HPV types by HPV-16/18 vaccination has not been shown in other clinical trials of HPV vaccines. Results of studies of natural HPV infection have shown type-specific response with some serological cross reactivity between phylogenetically related types.[21–23] Besides serological cross reactivity, another possible immune mechanism is the cell-mediated T-helper cell response.[24] Additional data and analyses are needed to ascertain the importance of cross-protection on lesion development and to establish an understanding of the mechanisms that underlie cross protection.

In conclusion, immunisation with the HPV-16/18 L1 virus-like particle vaccine adjuvanted with AS04 induces sustained high levels of antibodies that provide protection against HPV-16 and HPV-18 associated endpoints for up to 4·5 years. These findings set the stage for the widescale adoption of HPV vaccination for prevention of cervical cancer.

Contributors

D M Harper, E L Franco, C M Wheeler, A B Moscicki, B Romanowski, C M Rotelli-Martins, D Jenkins, A Schuind, S A Costa Clemens, and G Dubin contributed towards acquisition of data or statistical analyses, and/or interpretation of data, and writing and revision of the manuscript.

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Conflict of interest statement
D Jenkins, A Schuind, S A Costa Clemens, and G Dubin are employees of GlaxoSmithKline Biologicals. D M Harper has research contracts with Merck & Co, and 3M, and serves on their advisory boards. E L Franco has served as a consultant to GlaxoSmithKline and to other biotechnology and pharmaceutical companies that have products related to HPV diagnostics and vaccination. C M Wheeler has research contracts with Merck &Co. A-B Moscicki serves on the advisory board for GlaxoSmithKline. B Romanowski has received research grants from GlaxoSmithKline, Pfizer, Roche, and Schering; is a member of the advisory boards of GlaxoSmithKline, Novartis, and Roche; and is a member of the speakers' bureaus for GlaxoSmithKline, 3M, and Roche. C M Roteli-Martins declares she has no conflict of interest.

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