Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial

*Diane M Harper, Eduardo L Franco, Cosette Wheeler, Daron G Ferris, David Jenkins, Anne Schuind, Toufik Zahaf, Bruce Innis, Paulo Naud, Newton S De Carvalho, Cecilia M Roteli-Martins, Julio Teixeira, Mark M Blatter, Abner P Korn, Wim Quint, Gary Dubin, for the GlaxoSmithKline HPV Vaccine Study Group**

Lancet 2004; 364: 1757–65

*Contributors listed at end of paper

Departments of Obstetrics and Gynecology and Community and Family Medicine, Norris Cotton Cancer Center, Dartmouth Medical School, Hanover, NH, USA (D M Harper MD); **Department of Oncology and Epidemiology, McGill University, Montreal, Canada** (E L Franco PhD); **Departments of Molecular Genetics and Microbiology and Obstetrics and Gynecology, University of New Mexico Health Sciences Center, Albuquerque, NM, USA** (C Wheeler PhD); **Department of Family Medicine and Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA, USA** (D G Ferris MD); **GlaxoSmithKline Biologicals, Rixensart, Belgium** (D Jenkins MD, T Zahaf PhD); **GlaxoSmithKline Biologicals, King of Prussia, PA, USA** (A Schuind MD, B Innis MD, G Dubin MD); **Universidade Federal do Rio Grande do Sul, Porto Alegre Clinical Research Center for Vaccine Study, Porto Alegre, Brazil** (P Naud MD); **Department of Gynecology and Obstetrics, Ginecol Obstetric Infectious Diseases Sector, University of Parana, Curitiba, Parana, Brazil** (N S De Carvalho MD); **LM de Barros Hospital, Sao Paulo, Brazil** (C M Roteli-Martins MD); **Department of Gynecology, Oncology Division—CAISM, State University of Campinas, Campinas, Brazil** (J Teixeira MD); **Primary Physicians Research, Pittsburgh, PA, USA** (M M Blatter MD); **Department of Gynecology, University of California at San Francisco, San Francisco, USA** (A P Korn MD); **and Delft Diagnostic Laboratory, Delft, Netherlands** (W Quint PhD)

Correspondence to:
Dr Diane M Harper, Norris Cotton Cancer Center, Dartmouth Medical School, Gynecologic Cancer Prevention Research Group, Rubin 880, One Medical Center Drive, Lebanon, NH 03756, USA
diane.m.harper@dartmouth.edu

Summary

Background **Vaccination against the most common oncogenic human papillomavirus (HPV) types, HPV-16 and HPV-18, could prevent development of up to 70% of cervical cancers worldwide. We did a randomised, double-blind, controlled trial to assess the efficacy, safety, and immunogenicity of a bivalent HPV-16/18 L1 virus-like particle vaccine for the prevention of incident and persistent infection with these two virus types, associated cervical cytological abnormalities, and precancerous lesions.**

Methods **We randomised 1113 women between 15–25 years of age to receive three doses of either the vaccine formulated with AS04 adjuvant or placebo on a 0 month, 1 month, and 6 month schedule in North America and Brazil. Women were assessed for HPV infection by cervical cytology and self-obtained cervicovaginal samples for up to 27 months, and for vaccine safety and immunogenicity.**

Findings **In the according-to-protocol analyses, vaccine efficacy was 91·6% (95% CI 64·5–98·0) against incident infection and 100% against persistent infection (47·0–100) with HPV-16/18. In the intention-to-treat analyses, vaccine efficacy was 95·1% (63·5–99·3) against persistent cervical infection with HPV-16/18 and 92·9% (70·0–98·3) against cytological abnormalities associated with HPV-16/18 infection. The vaccine was generally safe, well tolerated, and highly immunogenic.**

Interpretation **The bivalent HPV vaccine was efficacious in prevention of incident and persistent cervical infections with HPV-16 and HPV-18, and associated cytological abnormalities and lesions. Vaccination against such infections could substantially reduce incidence of cervical cancer.**

Introduction

Cervical cancer is the most important manifestation of genital human papillomavirus (HPV) infection and is one of the leading causes of cancer mortality in women worldwide. The global disease burden of cervical cancer is estimated at 470 000 new cases and 230 000 deaths every year; almost 80% of the cases occur in developing countries, where in many regions it is the most common cancer among women.[1,2] Cervical cancer is also the leading cause of years-of-life-lost in women in south central Asia, Latin America, and sub-Saharan Africa, results in a greater reduction of a woman's life expectancy compared with AIDS, tuberculosis, or maternal conditions in Latin America and Europe.[3]

The causal role of some high-risk HPV types in cervical carcinogenesis has now been clearly established by studies that take into account the many molecular, epidemiological, virological, cytological, and histological complexities of the disease's natural history. Molecular studies show high-risk HPV DNA has been detected in 99·7% of an international series of cervical cancers with highly sensitive PCR, and, in 100% of cases, confirmed by expert histological review.[4]

The odds ratio for cervical cancer associated with high-risk HPV infection has been estimated as greater than 150 in case-control studies.[5] Findings from case-control studies and cohort studies together with laboratory evidence of HPV oncogenic expression, have established that persistent infection with high-risk HPV types is the necessary cause of cervical cancer.[6–9] The most prevalent HPV types associated with cervical cancer are HPV-16 and HPV-18; HPV-16 accounts for more than 60% of cervical cancers, with HPV-18 adding about another 10%.[5,10–13]

HPV vaccines based on L1 virus-like particles have shown promise in protecting against infection and development of lesions.[14,15] Recently, a monovalent HPV-16 virus-like particle vaccine showed protection against persistent infection with HPV-16 and its associated cervical intraepithelial neoplasia (CIN).[16] These data suggest that L1 virus-like particle vaccines have the potential to reduce worldwide cervical cancer rates.

We did a double blind, multi-centre, randomised, placebo-controlled clinical trial to assess the efficacy of a bivalent HPV-16/18 virus-like particle vaccine against incident and persistent infections with HPV-16 and HPV-18. We also assessed vaccine efficacy against cytological abnormalities and CIN, and vaccine immunogenicity, safety, and tolerability.

Methods

Study objectives and participants

The primary objective of this study was to assess vaccine efficacy in the prevention of infection with HPV-16, HPV-18, or both (HPV-16/18), between months 6 and 18 in participants who were initially shown to be seronegative for HPV-16/18 by ELISA and negative for HPV-16/18 DNA by PCR. Secondary objectives included: evaluation of vaccine efficacy in the prevention of persistent infection with HPV-16/18, and the evaluation of vaccine efficacy in the prevention of cytologically confirmed low-grade squamous intra-epithelial lesions (LSIL), high-grade squamous intra-epithelial lesions (HSIL), and histologically confirmed LSIL (CIN 1), HSIL (CIN 2 or 3) squamous cell cancer, or adenocarcinoma associated with HPV-16/18 infection between months 6 and 18, and months 6 and 27. The prevention of atypical squamous cells of undetermined significance (ASCUS) cytology associated with HPV-16/18 infection was added post-hoc to the outcome analyses.

We also did an exploratory analysis of the histopathological endpoints CIN 1 and 2 associated with HPV-16/18 DNA detected by PCR in lesional tissue. Other objectives included the assessment of vaccine immunogenicity, safety, and tolerability.

Investigators in North America (Canada and the USA) and Brazil recruited women for this efficacy study through advertisements or previous participation in an HPV cross-sectional epidemiology study that took place between July and December, 2000.

For each of the 32 study sites, an institutional review board approved the protocol, consent forms, and amendments. Women signed separate written consents for study participation and colposcopy. For those under 18 years, parental consent and assent from the participant were obligatory.

There were two study phases: an initial phase for vaccination and follow-up that concluded at month 18; and a blinded follow-up extension phase that concluded at month 27.

Women eligible for the initial phase (months 0–18) included healthy women aged 15–25 years, who had had no more than six sexual partners, no history of an abnormal Pap test or ablative or excisional treatment of the cervix, and no ongoing treatment for external condylomata; and who were cytologically negative, seronegative for HPV-16 and HPV-18 antibodies by ELISA, and HPV-DNA-negative by PCR for 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) no more than 90 days before study entry.

Women who completed the initial phase of the study earliest, and who did not have ablative or excisional therapy of the cervix, or hysterectomy after enrolment, were eligible to participate in the extension phase of the study (months 18–27).

Procedures

Each dose of the bivalent HPV-16/18 virus-like particle vaccine (GlaxoSmithKline Biologicals, Rixensart, Belgium) contained 20 μg of HPV-16 L1 virus-like particle and 20 μg of HPV-18 L1 virus-like particle. Each type of virus-like particle was produced on *Spodoptera frugiperda* Sf-9 and *Trichoplusia ni* Hi-5 cell substrate with AS04 adjuvant containing 500 μg aluminum hydroxide and 50 μg 3-deacylated monophosphoryl lipid A (MPL, Corixa, Montana, USA) provided in a monodose vial. The placebo contained 500 μg of aluminum hydroxide per dose, and was identical in appearance to the HPV-16/18 vaccine. Every study participant received a 0·5 mL dose of vaccine or placebo at 0 months, 1 month, and 6 months.

Health-care providers obtained cervical specimens with a cervical brush and spatula (washed in PreservCyt, Cytyc Corporation, Boxborough, MA, USA) for cytology and HPV DNA testing at screening and months 6, 12, and 18. At months 0 and 6, and subsequently every 3 months, women self-obtained cervicovaginal samples with two sequential swabs (placed in PreservCyt) for HPV DNA testing.[17] A central laboratory (Quest Diagnostics, Teterboro, NJ, USA) reported cytology results (ThinPrep, Cytyc Corporation) by use of the 1991 Bethesda classification system.

Protocol guidelines recommended colposcopy after two reports of ASCUS, or one report of atypical glandular cells of undetermined significance, LSIL or HSIL, squamous cell carcinoma, adenocarcinoma in situ, or adenocarcinoma. These guidelines also recommended biopsy for any suspected lesions.

The central histology laboratory made an initial diagnosis from the formalin-fixed tissue specimens for clinical management. A panel of three pathologists made a subsequent consensus diagnosis for HPV-16 and HPV-18 associated lesions with the CIN system. This consensus diagnosis also included review of the sections taken at the time of microdissection for PCR detection of lesional HPV DNA.

HPV DNA isolated from the cytology specimen (MagNaPure Total Nucleic Acid system, Roche Diagnostics, Almere, Netherlands) and from the cervical biopsy specimen (proteinase K extraction) was amplified from an aliquot of purified total DNA with the SPF10 broad-spectrum primers that amplify a 65 bp region of the L1 gene.[18–20] The amplification products were detected by a DNA enzyme immunoassay. A line probe assay (LiPA Kit HPV INNO LiPA HPV genotyping assay, SPF-10 system version 1, Innogenetics, Gent, Belgium, manufactured by Labo Bio-medical Products, Rijswijk, Netherlands) detected 25 HPV genotypes (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, and 74).[21] Any specimen that was positive by DNA enzyme immunoassay was tested by type-specific HPV-16 and HPV-18 PCR. HPV-16 type-specific PCR primers amplified a 92 bp segment of the E6/E7 gene

and HPV-18 type-specific PCR primers amplified a 126 bp segment of the L1 gene.[22]

We defined incident cervical infection with HPV-16/18 as at least one positive PCR result for HPV-16 or HPV-18 during the trial, and persistent infection with HPV-16/18 as at least two positive HPV-DNA PCR assays for the same viral genotype separated by at least 6 months.[23,24] HPV-DNA test results were concealed from investigators during the study and cytological and histological diagnoses were only revealed for clinical management purposes. Analyses included HPV-16/18 DNA results for cervical specimens and combined cervical and self-obtained cervicovaginal specimens.

We collected serum from study participants at months 0, 1, 6, 7, 12, and 18 for assessment of immunogenicity. Serological testing for antibodies to HPV-16 and HPV-18 virus-like particles was by ELISA. Recombinant HPV-16 or HPV-18 virus-like particles were used as coating antigens for antibody detection (see webappendix http://image.thelancet.com/extras/04art10103webappendix.pdf). Seropositivity was defined as a titre greater than or equal to the assay cut-off titre established at 8 ELISA units/mL for HPV-16 and 7 ELISA units/mL for HPV-18. Typical natural titres were determined by use of blood samples obtained from women in the preceding epidemiology study who were found to be seropositive for HPV-16 or HPV-18 by ELISA.

Women recorded symptoms experienced during the first 7 days after vaccination on diary cards with a three-grade scale of symptom intensity. Additionally, they reported to study personnel by interview all adverse events within the first 30 days after vaccination. Information on serious adverse events and pregnancies was collected throughout the study.

Statistical methods

Assuming a 6% cumulative incidence rate of both HPV-16 and HPV-18 type infections over 12 months, we estimated that 500 women per treatment group would provide 80% power to assess a lower limit of the 95% CI of the vaccine efficacy above zero. We assumed an 80% retention rate over 18 months. Interim analyses for efficacy, safety, and immunogenicity were done for future study planning purposes only; the O'Brien and Fleming method was used to adjust the α value for the final analysis after interim analyses occurred (overall $\alpha=0\cdot05$; two-sided test).[25]

Stratified, block randomisation according to validated algorithms was centralised with an internet randomisation system. Stratification was according to age (15–17, 18–21, and 22–25 years) and region (North America and Brazil). Each vaccine dose was attributed a randomly chosen number based on specific participant information entered into the computerised randomisation system by study personnel. Treatment allocation remains concealed from investigators and the women participating in a long-term follow-up study.

The intention-to-treat and according-to-protocol cohorts are shown in the figure, in which the reasons for exclusion from analyses are listed in rank order; women who met more than one exclusion criterion were only counted once according to the highest ranking criterion. We refer to the sets of participants entered in the intention-to-treat and according-to-protocol analyses as cohorts, although the information used to restrict subject inclusion in the according-to-protocol was only known after follow-up.

We did both according-to-protocol and intention-to-treat analyses for efficacy. Calculation of vaccine efficacy in the according-to-protocol 18-month analysis was based on the proportion of participants with HPV-16/18 infection in the vaccinated versus placebo groups. Vaccine efficacy was defined as 1 minus the ratio between these two proportions; 95% CIs measured the precision of the efficacy estimates. p values were calculated with the two-sided Fisher's exact test. Corresponding rates were expressed as the numbers of cases with the outcome divided by the numbers of participants at risk. The according-to-protocol 18-month cohort included enrolled women who received three scheduled doses of vaccine and complied with the protocol as described in the figure.

Calculation of vaccine efficacy in the intention-to-treat and according-to-protocol 27-month analyses was based on the Cox proportional hazard model using the time-to-occurrence of cases with HPV-16/18 infection in the vaccinated versus placebo groups. This allowed controlling for the accrued person-time data in each group. Vaccine efficacy was calculated using 1 minus the hazard ratio and p values calculated using the log rank test. Corresponding rates were expressed as the number of cases divided by the total person-time. All enrolled women who received at least one dose of vaccine or placebo, were negative for high-risk HPV-DNA at month 0, and had any data available for outcome measurement were included in the intention-to-treat cohort. The according-to-protocol 27-month cohort included outcome results from the according-to-protocol 18-month cohort and results that occurred during the extension phase (from 18 months to 27 months).

Calculation of p values for the safety analysis was performed using Fisher's exact test comparisons. The cohort for safety analysis included all enrolled women who received at least one dose of vaccine or placebo and complied with specified, minimal protocol requirements (figure).

Immunogenicity was assessed in a subset of the according-to-protocol safety cohort, which included women with serology results at months 0, 7, and 18, who received all three doses of study vaccine or placebo according to schedule, complied with the blood sampling schedule, and did not become positive for HPV-16/18-DNA during the trial (figure). Seropositivity rates between the vaccine and placebo groups were compared with Fisher's exact test

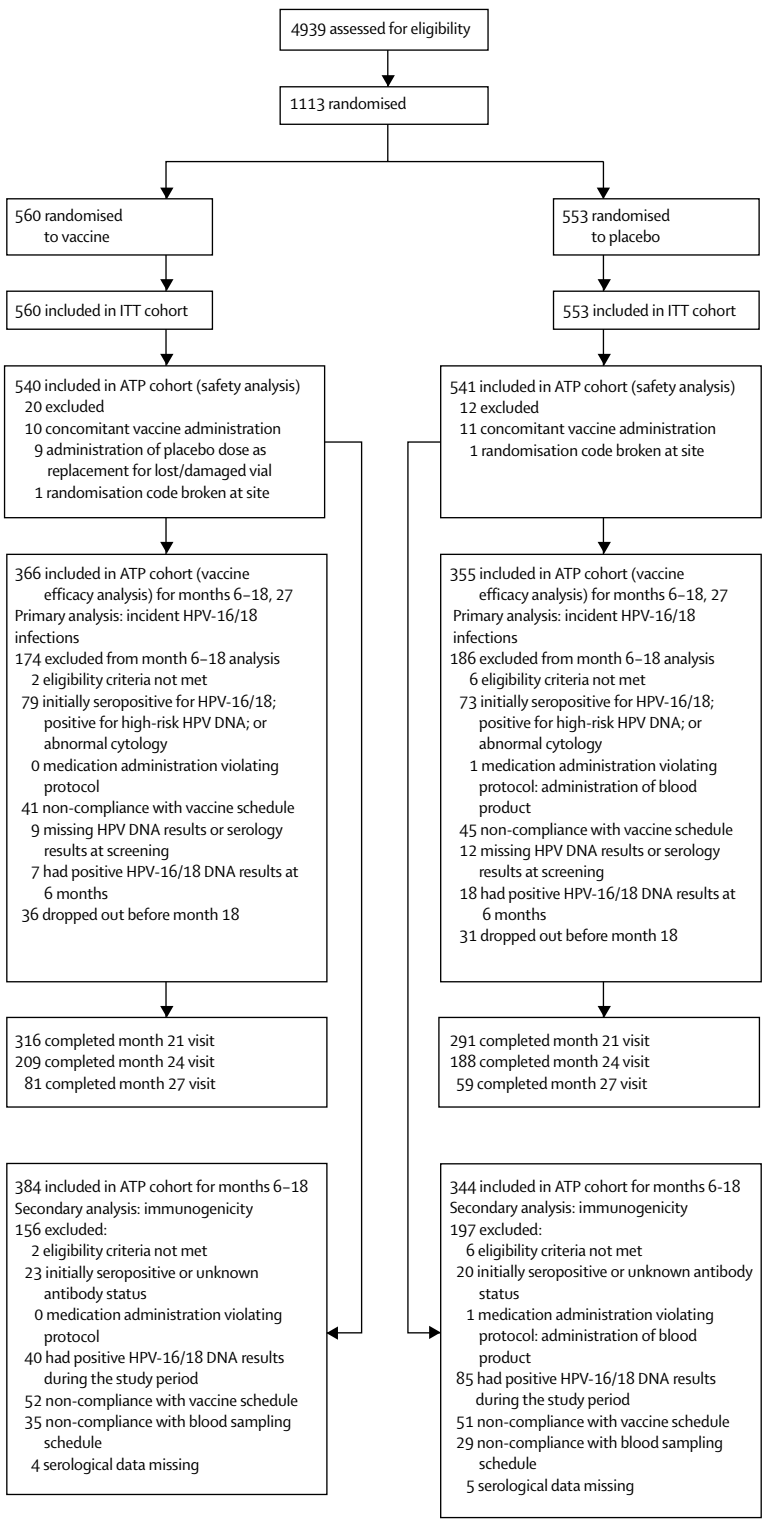

Figure: **Trial profile**

	ITT cohort*		ATP cohort*	
	Vaccine group (n=560)	Placebo group (n=553)	Vaccine group (n=366)	Placebo group (n=355)
Age (years)	20·4 (2·8)	20·5 (2·7)	20·2 (2·9)	20·5 (2·8)
Region				
North America†	302 (54·0%)	305 (55·2%)	181 (49·5%)	182 (51·3%)
Brazil	258 (46·0%)	248 (44·8%)	185 (50·5%)	173 (48·7%)
Ethnic group				
White	389 (69·5%)	384 (69·4%)	244 (66·7%)	249 (70·1%)
Black	43 (7·7%)	41 (7·4%)	30 (8·2%)	22 (6·2%)
Asian	9 (1·6%)	4 (0·7%)	9 (2·5%)	4 (1·1%)
Other	119 (21·3%)	124 (22·4%)	83 (22·7%)	80 (22·5%)

Data are mean (SD) for age and number (%) for other values. Percentages may not add up to 100% exactly due to rounding. ITT=intention-to-treat. ATP=according-to-protocol (18-month cohort for efficacy). *No demographic differences between groups within cohorts or between cohorts (Fisher's exact test). †Includes Canada and the USA.

Table 1: **Demographic characteristics by analysis cohort and treatment group**

(p<0·001 judged significant). Geometric mean titres were compared with ANOVA and Kruskal-Wallis test.

Block randomisation and statistical analyses were done with SAS version 8.2 (SAS Institute, Cary, North Carolina).

Role of the funding source

This study was conceived jointly by GlaxoSmithKline Biologicals and consultants, some of whom also served as investigators. GlaxoSmithKline Biologicals funded and coordinated this study. A publication steering committee was assembled to represent all members of the HPV Vaccine Study group who collected data for the study and cared for the study patients. GlaxoSmithKline Biologicals did all HPV serological testing, Quest Diagnostics processed all cytology and histology specimens, and Delft Diagnostic Laboratory did PCR for HPV types. An independent external statistician worked separately on data analyses to maintain allocation concealment; and the sponsor drafted the clinical study report for regulatory purposes with these data provided. The corresponding author had full access to the data and had final responsibility for submission for publication.

	Vaccine group (n=560)	Placebo group (n=553)
Compliance with vaccinations*		
Dose 1	560 (100·0%)	553 (100·0%)
Dose 2	540 (96·4%)	536 (96·9%)
Dose 3	523 (93·4%)	513 (92·8%)
Study visit completion		
Complete to month 18	480 (85·7%)	478 (86·4%)
Dropouts†	80 (14·3%)	75 (13·6%)
Women lost to follow-up		
With complete vaccination course	18 (3·2%)	24 (4·3%)
With incomplete vaccination course	15 (2·7%)	11 (2·0%)

*Number of women receiving each dose (% doses received); other data are number (%). †Women who did not come for the visit at month 18.

Table 2: **Study compliance characteristics for women included in the intention-to-treat cohort**

	HPV type	Follow-up (months)	Vaccine group			Placebo group			Vaccine efficacy (95%CI)	p
			Cases	Episodes	Rate	Cases	Episodes	Rate		
ATP cohort (vaccine n=366, placebo n=355)										
Cervical samples	16	18	0	0	0·0%	18	25	5·1%	100% (79·4 to 100·0)	<0·0001
	18	18	2	2	0·6%	7	7	2·0%	72·3% (−32·5 to 93·4)	0·102
	16/18	18	2	2	0·6%	23	30	6·5%	91·6% (64·5 to 98·0)	<0·0001
Combined cervicovaginal and cervical samples	16	27	6	6	0·9%	29	77	4·6%	81·2% (54·8 to 92·2)	<0·0001
	18	27	6	6	0·9%	16	27	2·5%	65·1% (10·8 to 86·3)	0·021
	16/18	27	12	12	1·8%	41	97	6·6%	73·6% (49·7 to 86·1)	<0·0001
ITT cohort (vaccine n=560, placebo n=553)										
Cervical sample	16	27	5	7	0·7%	30	50	4·6%	82·7% (55·2 to 93·3)	<0·0001
	18	27	3	3	0·4%	17	23	2·6%	82·1% (38·8 to 94·7)	0·002
	16/18	27	7	9	1·0%	42	68	6·5%	83·0% (62·0 to 92·4)	<0·0001
Combined cervicovaginal and cervical samples	16	27	14	24	1·7%	53	154	6·9%	75·2% (55·3 to 86·2)	<0·0001
	18	27	13	18	1·6%	31	67	3·9%	59·5% (22·7 to 78·8)	0·005
	16/18	27	25	39	3·1%	72	196	9·5%	67·6% (48·9 to 79·4)	<0·0001

See Methods for calculations of vaccine efficacy, corresponding rates, and p values. Cases=number of women. ITT=intention-to-treat. ATP=according-to-protocol. HPV-16/18=a single instance of HPV-16 or HPV-18 as single or combined infection. Episodes=number of times HPV type was detected.

Table 3: **Vaccine efficacy for incident HPV-16/18 infections**

Results

Based on the estimate of appropriate study sample size, 1113 women were enrolled and randomised. We administered the study vaccine to 560 women and the placebo to 553 women (figure). The average age of enrolled women was 20 years (SD 3). The demographic characteristics were similar between the vaccine and placebo groups in North America and Brazil (table 1; webtable 1 at http://image.thelancet.com/extras/04art10103webtable1.pdf). We noted similar patterns of risk factors for HPV acquisition among women in each treatment group: about half of the women were current smokers, a large proportion had between two and five previous sexual partners, and most began sexual activity between 15 years and 19 years of age (see webtable 2 at http://image.thelancet.com/extras/04art10103webtable2.pdf).

Study compliance is shown in table 2. The major reasons for elimination from according-to-protocol efficacy analysis were abnormal cytology, high-risk HPV DNA positivity, or seropositivity for HPV-16 or HPV-18 at enrolment; followed by non-compliance with the vaccine schedule and drop-out from the study up to month 18 (figure, table 2). 958 women (85%) completed the initial phase to month 18, with similar proportions of women from the vaccine and placebo dropping out of the study (table 2).

Analyses to assess our primary objective showed significant vaccine efficacy against incident HPV-16 and HPV-16/18 infections (table 3). In the according-to-protocol 18-month cohort, vaccine efficacy against incident HPV-18 infection was not statistically significant; however, in the intention-to-treat cohort, vaccine efficacy was significant (analysis of cervical samples only).

We noted 100% vaccine efficacy in the according-to-protocol cohorts against persistent HPV-16 and HPV-16/18 infections detected in both cervical and combined cervical and cervicovaginal samples (table 4). Although no

	HPV type	Follow-up (months)	Vaccine group		Placebo group		Vaccine efficacy (95% CI)	p
			Cases	Rate	Cases	Rate		
ATP cohort (vaccine n=366, placebo n=355)								
Cervical samples	16	18	0	0·0%	7	2·0%	100% (47·0–100)	0·007
	18	18	0	0·0%	0	0·0%	NC	..
	16/18	18	0	0·0%	7	2·0%	100% (47·0–100)	0·007
Combined cervicovaginal and cervical samples	16	27	0	0·0%	13	2·1%	100% (71·5–100)	0·0002
	18	27	0	0·0%	4	0·6%	100% (7·2–100)	0·040
	16/18	27	0	0·0%	16	2·6%	100% (76·8–100)	<0·0001
ITT cohort (vaccine n=560, placebo n=553)								
Cervical samples	16	27	1	0·1%	16	2·5%	93·9% (53·2–99·2)	0·0002
	18	27	0	0·0%	5	0·8%	100% (24·4–100)	0·025
	16/18	27	1	0·1%	20	3·1%	95·1% (63·5–99·3)	<0·0001
Combined cervicovaginal and cervical samples	16	27	4	0·5%	25	3·2%	84·5% (55·2–94·6)	<0·0001
	18	27	1	0·1%	11	1·4%	91·1% (31·0–98·9)	0·003
	16/18	27	4	0·5%	31	4·0%	87·5% (64·6–95·6)	<0·0001

See Methods for calculations of vaccine efficacy, corresponding rates, and p values. Cases=number of women. ITT=intention-to-treat. ATP=according-to-protocol. HPV-16/18 refers to two or more instances of HPV-16 or HPV-18 infection or combined infections regardless of other HPV types over a minimum period of 6 months. NC=no cases reported, vaccine efficacy not estimated.

Table 4: **Vaccine efficacy for persistent HPV16/18 infections**

HPV type associated with ⩾ASCUS	Vaccine group (n=560)			Placebo group (n=553)			Vaccine efficacy (95%CI)	p
	Cases	Episodes	Rate	Cases	Episodes	Rate		
16	1	2	0·2%	20	26	3·6%	95·2% (64·0–99·4)	<0·0001
18	1	1	0·2%	11	12	2·0%	91·2% (31·7–98·9)	0·003
16/18	2	3	0·4%	27	33	4·9%	92·9% (70·0–98·3)	<0·0001

See Methods for calculations of vaccine efficacy, corresponding rates, and p values. ITT=intention-to-treat. HPV-16/18 refers to instances of HPV-16 and/or HPV-18 as single or combined infections at the time of abnormal cervical smear; HPV-16 refers to the presence of HPV-16 irrespective of other HPV types present; and HPV-18 indicates the presence of HPV-18 irrespective of other HPV types present. ⩾ASCUS includes women who had HPV-16/18-associated ASCUS, LSIL, or HSIL cytology reports. Episodes=number of times abnormal cytology was detected. Cases=number of women.

Table 5: **Vaccine efficacy in preventing cytological abnormalities associated with HPV-16/18 infection (intention-to-treat cohort)**

persistent HPV-18 infections were detected in cervical samples from the according-to-protocol 18-month cohort, significant vaccine efficacy against persistent HPV-18 infection was shown in the 27-month cohort for combined cervical and cervicovaginal samples. Additionally, significant vaccine efficacy against persistent HPV-16 and HPV-16/18 infections was noted in the intention-to-treat cohort in analyses of cervical samples only and of combined cervical and cervicovaginal samples.

As shown in table 5, 27 women in the placebo group and two in the vaccine group had HPV-16 and/or HPV-18 associated cytological abnormalities (vaccine group: one woman with ASCUS, and one with LSIL). 15 ASCUS, 14 LSIL, and one HSIL were reported in the placebo group and one ASCUS, two LSIL, and no HSIL in the vaccine group. Vaccine efficacy in the according-to-protocol cohort was 93·5% (95% CI 51·3–99·1; p=0·0002).

We also assessed women with histologically confirmed CIN 1 or 2 lesions, with HPV-16 or HPV-18 infection detected in the cytology specimen before colposcopy. Overall, seven women (six in the placebo group and one in the vaccine group), developed these lesions (table 6).

One woman in the vaccine group first tested positive for HPV-51 and HPV-56 at month 9. Her cytology specimen at month 12 tested positive for HPV types 18, 51, and 56. The CIN 1 lesion found in the colposcopically-directed

	Findings by sample type	Sample collection time frame (months)							
		6	9	12	15	18	21	24	27
1 (Vaccine)	Cytology/biopsy	..	..	LSIL/CIN 1	..	..	..	na	..
	HPV-16, 18 (cv/c)	..		ND/18		..	..	..	..
	Other HR HPV (cv/c)	..	51, 56/na	51, 56/51, 56	51/na	..	51/na	..	..
	Tissue (all HR HPV types)2	..	..	51	..	..	..	..	..
2 (Placebo)	Cytology/biopsy	..	..	ASCUS	..	LSIL/CIN 1	..	..	na
	HPV-16, 18 (cv/c)	..	..	16/16	16/na	16/16	16/na	16/na	..
	Other HR HPV (cv/c)	..	..	..	..	..	..	..	..
	Tissue (all HR HPV types)	..	..	..	..	16	..	..	..
3 (Placebo)	Cytology/biopsy	..	..	..	..	LSIL	CIN 1	..	..
	HPV-16, 18 (cv/c)	..	..	..	16/na	16/16	16/na	16/na	16/16
	Other HR HPV(cv/c)	39/39	39/na	39/39	..	..	6/na	..	..
	Tissue (all HR HPV types)	..	..	..	..	..	16	..	..
4 (Placebo)	Cytology/biopsy	..	..	..	..	LSIL/CIN 1	na	na	na
	HPV-16, 18 (cv/c)	..	..	16/16	16/na	16/16	..	..	..
	Other HR HPV(cv/c)	..	..	58/58	39, 58/na	58/58	..	..	..
	Tissue (all HR HPV types)	..	..	..	..	16, 58	..	..	..
5 (Placebo)	Cytology/biopsy	..	..	..	..	LSIL/CIN 2	..	na	na
	HPV-16, 18 (cv/c)	..	16/na	na/16	16/na	16	16/na	..	..
	Other HR HPV(cv/c)	..	..	..	..	..	..	..	..
	Tissue (all HR HPV types)	..	..	..	..	16	..	..	..
6 (Placebo)	Cytology/biopsy	..	..	ASCUS	..	LSIL/CIN 2	..	..	..
	HPV-16, 18 (cv/c)	16/16	16/na	16/16	16/na	16/16	16/na	..	..
	Other HR HPV (cv/c)	..	..	..	39/na	59, 74/39	39, 59, 74/na	51, 74/na	51, 74/na
	Tissue (all HR HPV types)	..	..	..	..	16	..	..	..
7 (Placebo)	Cytology/biopsy	..	..	HSIL/CIN 2	..	..	..	..	na
	HPV-16, 18 (cv/c)	16/16	16	16/16	16/na	..	..	..	..
	Other HR HPV (cv/c)	66, 68/66	66/na	..	..	39, 66/39, 66	90*/na	66/na	..
	Tissue (all HR HPV types)	..	..	16	..	..	..	..	..

Numbers are HPV types. Only positive HPV detection and abnormal cytology or histology results are shown.Months with no results indicate negative HPV results. Cytology specimens were obtained before the biopsy samples within the same time window. When the cytology and histology results are shown in the same time window, the cytology result preceded the biopsy. For cytohistopathology findings, / separates cytology (left) from histology (right) results. For viral findings, / separates results based on self-obtained cervicovaginal samples (cv) or cervical cytology specimens (c). Women with abnormal cytology at month 18 might have had cervical sample obtained by physican at a subsequent visit. Tissue refers to the colposcopically directed biopsy sample. HR HPV=high-risk HPV. ND=not detected. na=data not available or sample not obtained at timepoint. *HPV-90 was detected by a different detection method than that used for the other high-risk HPV types (DNA sequencing).

Table 6: **Timeline of virological, cytological, and histological findings among the seven women with HPV-16/18-associated abnormal cytology that was histologically confirmed as CIN (intention-to-treat cohort)**

biopsy contained only HPV-51, and not HPV-18. Subsequent HPV testing showed persistent HPV-51 through month 21; therefore, the lesion was finally judged to be associated with HPV-51.

Three women who received placebo developed CIN 1; all had ASCUS or LSIL cytology and a pattern of persistent HPV-16 infection. HPV-16 was detected in the lesions of all three women. Two women remained HPV-16 positive after biopsy and the third woman had a persistent HPV-58 infection in addition to persistent HPV-16 infection.

Consensus diagnosis confirmed CIN 2 diagnoses in three other women in the placebo group with preceding ASCUS, LSIL, and HSIL cytological abnormalities. In each case, HPV-16 was detected in all lesions and persistent HPV-16 infections preceded the lesions.

No serious adverse events related to vaccination occurred in either vaccine or placebo groups. The vaccine group had more injection site symptoms (pain, swelling, redness) than the placebo group (table 7; overall injection site symptoms 5·9% difference between proportions, 95% CI 2·1–10·1, Fisher's exact test), but these symptoms tended to be transient and mild. The difference in incidence of injection site reactions between the groups had no effect on compliance with completion of the vaccination course (table 2). The general symptoms of fatigue, gastrointestinal complaints, headache, itching, and rash were equally common in both the vaccine and placebo groups. Three women in the placebo group dropped out because of non-serious adverse events; one woman in the vaccine group dropped out because of a serious adverse event (spontaneous abortion) that was not related to vaccination (figure, table 7).

Among the vaccinated women in the according-to-protocol cohort from month 0 to month 7, 100% seroconverted to HPV-16-positive and 99·7% seroconverted to HPV-18-positive after three doses of vaccine (table 8). By 18 months, 100% of the women had seroconverted for both HPV-16 and HPV-18. Comparison of GMTs at month 7 between vaccine and placebo groups for each antibody type was significant (p<0·0001).

Geometric mean titres for naturally occurring infections were 50 ELISA units/mL (SD 0·5, 95% CI 40·9–60·4) for antibodies against HPV-16 and 41 ELISA units/mL (0·5, 34·2–49·0) for antibodies against HPV-18. Geometric mean titres for vaccine-induced antibodies to HPV antibodies were over 80 and 100 times greater than those seen in natural infections with HPV-18 and HPV-16, respectively. Vaccine-induced titres remained substantially raised at 18 months, and were still 10–16 times higher than those seen in women with natural HPV-16 or HPV-18 infections, respectively.

	Vaccine group (n=531)	Placebo group (n=538)	p
Serious adverse events			
Related to vaccination	0	0	..
During study*	22 (4·0%)	19 (3·5%)	0·636
Injection site symptoms†			
Pain	496 (93·4%)	469 (87·2%)	0·0006
Swelling	182 (34·3%)	113 (21·0%)	<0·0001
Redness	189 (35·6%)	131 (24·3%)	0·0001
Overall‡	499 (94·0%)	472 (87·7%)	0·0004
General symptoms†			
Fatigue	308 (58·0%)	289 (53·7%)	0·175
Gastrointestinal	178 (33·5%)	172 (32·0%)	0·602
Headache	331 (62·3%)	329 (61·2%)	0·706
Itching	130 (24·5%)	109 (20·3%)	0·106
Rash	60 (11·3%)	54 (10·0%)	0·552
Raised temperature§	88 (16·6%)	73 (13·6%)	0·172
Overall‡	458 (86·3%)	462 (85·9%)	0·860
Withdrawal from study			
Due to non-serious adverse event	0	3 (0·6%)	0·249
Due to serious adverse event	1 (0·1%)	0	0·497

Data represent women in the according-to-protocol safety cohort who returned a diary card or reported an unsolicited symptom. Data are number (%) of participants. *Participants who reported a serious adverse event during the entire study period (month 0–27).†Participants who reported a specified symptom within 7 days of vaccine injection. ‡Participants who presented with at least one type of symptom within 30 days of vaccine injection. §Defined as oral temperature >37·5°C; no temperatures of >39·0°C were reported.

Table 7: **Adverse events after any vaccine dose in according-to-protocol cohort for safety**

Discussion

The results of this trial show that the bivalent HPV-16/18 virus-like particle vaccine was highly efficacious in preventing incident and persistent HPV-16/18 infection in fully vaccinated healthy young women. The vaccine was also highly efficacious in the broader group of women, including those who did not fully comply with the protocol.

The incidence of HPV-16 infection in the placebo group was sufficient to detect significant differences compared with the vaccine arm in all cohorts. However, the incidence of HPV-18 was much lower, as noted in other studies.[26] Nonetheless, significant results for vaccine efficacy against HPV-18 were obtained in the intention-to-treat cohort, where there were sufficient numbers of events for analysis. We conclude that the bivalent vaccine shows high efficacy against both incident and persistent HPV-16 and HPV-18 infections.

Incident HPV infection was common in young women in this study, as noted in previous cohort studies.[27] Because we used two PCR methods (broad-primer testing and type-specific testing) designed for maximum sensitivity to detect HPV-16/18, many incident infections were detected only on one occasion, particularly in cervicovaginal samples. It is likely that this single timepoint detection represents the presence of very small amounts of HPV DNA, possibly as a result of HPV presence not related to active infection or very low-grade transient infection.[28]

We provide evidence for the close relation between the development of persistent HPV infection and the concomitant development of cytological abnormalities, followed by the detection of CIN from biopsy. These results are consistent with those of previous epidemiological cohort studies of the natural history of HPV infection. Compared with the subjective readings of

	Time	n	Total number of seropositive individuals (%, 95% CI)	Geometric mean titre in ELISA units/mL (95% CI)
Anti-HPV-16				
Vaccine group	Enrolment	352	0 (0%, 0·0–1·0)	4·0 (4·0–4·0)
	Month 7	351	351 (100·0%, 99·0–100·0)	5334·5 (4766·9–5969·6)
	Month 18	348	348 (100·0%, 98·9–100·0)	801·4 (706·4–909·2)
Placebo group	Enrolment	310	0 (0%, 0·0–1·2)	4·0 (4·0–4·0)
	Month 7	310	10 (3·2%, 1·6–5·9)	4·2 (4·0–4·3)
	Month 18	310	10 (3·2%, 1·6–5·9)	4·2 (4·1–4·3)
Anti-HPV-18				
Vaccine group	Enrolment	352	0 (0%, 0·0–1·0)	3·5 (3·5–3·5)
	Month 7	351	350 (99·7%, 98·4–100·0)	3364·7 (3015·1–3754·9)
	Month 18	348	348 (100·0%, 98·9–100·0)	480·5 (424·5–543·8)
Placebo group	Enrolment	310	0 (0%, 0·0–1·2)	3·5 (3·5–3·5)
	Month 7	310	4 (1·3%, 0·4–3·3)	3·6 (3·5–3·7)
	Month 18	308	1 (0·3%, 0·0–1·8)	3·5 (3·5–3·6)

n=total number of individuals.

Table 8: **Anti-HPV antibody response to HPV-16 and HPV-18 virus-like particles (according-to-protocol cohort)**

cytology and cervical histopathology, detection of persistent infection with a type-specific HPV is a reliable endpoint with high reproducibility.[19] Previous studies have established the role of persistent HPV infection as the necessary cause of cervical cancer. Our data lend support to the conclusion that persistent type-specific infection with HPV over 6–12 months should be a recommended endpoint for vaccine efficacy trials.[29]

Taken together, our data provide compelling evidence that this HPV-16/18 vaccine is highly efficacious against persistent HPV-16/18 cervical infection, cytological abnormalities associated with HPV-16/18, and histological development of HPV-16/18-associated CIN. However, a limitation to our study was that it was not powered to estimate efficacy for histopathologically confirmed cervical lesions.

We have shown that the HPV-16/18 virus-like particle vaccine adjuvanted with AS04 induces a level of antibody production against HPV-16/18 that is much higher than that induced by natural infection. Previous work has shown that combinations of the adjuvants MPL and aluminum salts induce an enhanced immune response compared with antigen alone or adjuvanted with only aluminum, at both the humoral and cellular level.[30–32] These findings suggest that the immune responses induced in vaccinated women may provide a longer duration of protection than the protective effects induced by natural HPV infection; however, a protective antibody level has not been established nor is there sufficient data currently available to estimate the duration of vaccine-induced protection.

In this trial, the bivalent HPV-16/18 vaccine appeared to be safe and well tolerated. No serious vaccine-related adverse events were reported. Neither local nor general vaccine related symptoms affected overall subject compliance. Greater local reaction rates were observed in the vaccine group, but general symptom rates were equivalent to placebo. The AS04 adjuvant has been used in other vaccine studies and found to be generally safe and well tolerated.[32]

Our findings indicate that the vaccine could contribute substantially to reducing worldwide rates of cervical cancer. However, large-scale trials with long-term follow-up are needed to extend our findings and confirm that vaccination prevents cervical cancer.

Mathematical modelling predicts that a prophylactic vaccine programme, directed at young adolescent women, is likely to be cost-effective in both screened and unscreened populations, with important long-term implications for cervical cancer prevention, especially in countries where screening is limited or unavailable.[33–37] Additional benefits could come from vaccination of older women. In countries with opportunistic or organised screening programmes, the high vaccine efficacy in preventing cytological abnormalities associated with HPV-16/18 shows the potential to reduce the number of women receiving additional cytology or colposcopy, thereby reducing the cost of medical treatments associated with cervical screening programmes. In the USA, these preventable costs are estimated at several billion dollars per year.[38]

Further studies are in progress to provide additional information to enable the effective implementation of HPV vaccination as a public health measure aimed at reducing the global burden of cervical cancer.

HPV Vaccine Study Group

Publication steering committee

A Schuind, P Naud, C M Roteli-Martins, L B Twiggs, A P Korn, D Jenkins, G Dubin, B Romanowski (University of Alberta, Edmonton, Canada).

Principal investigators/co-investigators

Brazil (506 patients): P Colares de Borba, P Naud, CM Roteli-Martins, N S De Carvalho, J C Teixeira.

Canada (45 patients): F Aoki, B Ramjattan, B Romanowski, R M Shier, R Somani.

USA (562 patients): J Adelglass, S Barbier, M M Blatter, C Chambers, D G Ferris, S A Gall, M Gerardi, F Guerra, D M Harper, J Hedrick, D Henry, W Hooch, A P Korn, A B Moscicki, B Sullivan, C Thoming, L B Twiggs, S K Tyring, R Watson, C Wheeler, M Cabeszas-Mijuste.

Contributors

GlaxoSmithKline Biologicals (Rixensart, Belgium) in collaboration with E Connor and J Young from MedImmune (Gaithersburg, Maryland, USA), E L Franco, T Wright (Columbia University, NY, USA), A Lorincz (Digene Corporation, Gaithersburg, MD, USA), L B Twiggs (University

of Miami Medical School, Miami, FL, USA), C Wheeler, D G Ferris, and D M Harper contributed to the development of the study. D M Harper, E L Franco, C Wheeler, D G Ferris, D Jenkins, A Schuind, T Zahaf, B Innis, P Naud, N S De Carvalho, C M Roteli-Martins, J Teixeira, M M Blatter, A P Korn, W Quint, and G Dubin contributed towards acquisition or interpretation of data, and revision and final approval of the manuscript. R Luff (Quest Diagnostics, Teterboro, NJ, USA) contributed to cytology and histology results. W Quint, L-J van Doorn, and A Molijn (Delft Diagnostics Laboratory, Delft, Netherlands) did PCR LiPA testing and SPF Line blot type specific HPV testing. E L Franco provided contribution to the statistical plan and interpretation. External statistical analyses and support were provided by L Declerck and L Noiroux (S-Clinica, Brussels, Belgium). S L Wieting from GlaxoSmithKline Biologicals, Rixensart, Belgium, contributed to the technical writing aspects.

Conflict of interest statement

D Jenkins, A Schuind, T Zahaf, B Innis, and G Dubin are employees of GlaxoSmithKline Biologicals. E Franco has served as consultant on HPV and cervical cancer epidemiology to GlaxoSmithKline and to other biotechnology or pharmaceutical companies (3M, Digene, Cytyc) since 1999. D Ferris is a consultant for colposcopy quality control for GlaxoSmithKline Biologicals. M Blatter is a participant on speakers bureau and is doing research with GlaxoSmithKline, Aventis, MSD, Wyeth, and MedImmune. J Teixeira received research funding from GlaxoSmithKline Biologicals during the study. W Quint is an employee of Delft Diagnostic Laboratory, which did the molecular biological testing for this study. No other authors reported any conflicts of interest.

Acknowledgments

GlaxoSmithKline Biologicals funded and coordinated this study.

References

1 Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001; **94:** 153–56.

2 Parkin DM, Bray FI, Devesa SS. Cancer burden in the year 2000. The global picture. *Eur J Cancer* 2001; **37**(suppl 8): S4–66.

3 Yang BH, Bray FI, Parkin DM, Sellors JW, Zhang ZF. Cervical cancer as a priority for prevention in different world regions: an evaluation using years of life lost. *Int J Cancer* 2004; **109:** 418–24.

4 Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999; **189:** 12–19.

5 Munoz N, Bosch FX, De Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003; **348:** 518–27.

6 Bosch FX, De Sanjose S. Human papillomavirus in cervical cancer. *Curr Oncol Rep* 2002; **4:** 175–83.

7 Franco EL, Duarte-Franco E, Ferenczy A. Cervical cancer: epidemiology, prevention and the role of human papillomavirus infection. *CMAJ* 2001; **164:** 1017–25.

8 Schlecht NF, Platt RW, Negassa A, et al. Modeling the time dependence of the association between human papillomavirus infection and cervical cancer precursor lesions. *Am J Epidemiol* 2003; **158:** 878–86.

9 Bosch FX, De Sanjose S. Chapter 1: Human papillomavirus and cervical cancer—burden and assessment of causality. *J Natl Cancer Inst Monogr* 2003: 3–13.

10 Bosch FX, Manos MM, Munoz N, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. *J Natl Cancer Inst* 1995; **87:** 796–802.

11 Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *Br J Cancer* 2003; **88:** 63–73.

12 Clifford GM, Smith JS, Aguado T, Franceschi S. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. *Br J Cancer* 2003; **89:** 101–05.

13 Schiffman M, Castle PE. Human papillomavirus: epidemiology and public health. *Arch Pathol Lab Med* 2003; **127:** 930–34.

14 Harro CD, Pang YY, Roden RB, et al. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. *J Natl Cancer Inst* 2001; **93:** 284–92.

15 Schiller JT, and Lowy DR. Papillomavirus-like particle vaccines. *J Natl Cancer Inst Monogr* 2001: 50–4.

16 Koutsky LA, Ault KA, Wheeler CM, et al. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002; **347:** 1645–51.

17 Harper DM, Noll WW, Belloni DR, Cole BF. Randomized clinical trial of PCR-determined human papillomavirus detection methods: self-sampling versus clinician-directed—biologic concordance and women's preferences. *Am J Obstet Gynecol* 2002; **186:** 365–73.

18 Kleter B, van Doorn LJ, ter Schegget J, et al. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. *Am J Pathol* 1998; **153:** 1731–39.

19 van Doorn LJ, Quint W, Kleter B, et al. Genotyping of human papillomavirus in liquid cytology cervical specimens by the PGMY line blot assay and the SPF(10) line probe assay. *J Clin Microbiol* 2002; **40:** 979–83.

20 Quint WG, Scholte G, van Doorn LJ, Kleter B, Smits PH, Lindeman J. Comparative analysis of human papillomavirus infections in cervical scrapes and biopsy specimens by general SPF(10) PCR and HPV genotyping. *J Pathol* 2001; **194:** 51–58.

21 Kleter B, van Doorn LJ, Schrauwen L, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. *J Clin Microbiol* 1999; **37:** 2508–17.

22 Baay MF, Quint WG, Koudstaal J, et al. Comprehensive study of several general and type-specific primer pairs for detection of human papillomavirus DNA by PCR in paraffin-embedded cervical carcinomas. *J Clin Microbiol* 1996; **34:** 745–47.

23 Richardson H, Kelsall G, Tellier P, et al. The natural history of type-specific human papillomavirus infections in female university students. *Cancer Epidemiol Biomarkers Prev* 2003; **12:** 485–90.

24 Moscicki AB, Ellenberg JH, Farhat S, Xu J. Persistence of human papillomavirus infection in HIV-infected and -uninfected adolescent girls: risk factors and differences, by phylogenetic type. *J Infect Dis* 2004; **190:** 37–45.

25 O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics* 1979; **35:** 549–56.

26 Woodman CB, Collins S, Rollason TP, et al. Human papillomavirus type 18 and rapidly progressing cervical intraepithelial neoplasia. *Lancet* 2003; **361:** 40–43.

27 Wheeler CM, Greer CE, Becker TM, Hunt WC, Anderson SM, Manos MM. Short-term fluctuations in the detection of cervical human papillomavirus DNA. *Obstet Gynecol* 1996; **88:** 261–68.

28 Harper DM, Longacre MR, Noll WW, Belloni DR, Cole BF. Factors affecting the detection rate of human papillomavirus. *Ann Fam Med* 2003; **1:** 221–27.

29 Lowy DR, Frazer IH. Chapter 16: Prophylactic human papillomavirus vaccines. *J Natl Cancer Inst Monogr* 2003; **31:** 111–16.

30 Ulrich JT, Myers KR. Monophosphoryl lipid A as an adjuvant. Past experiences and new directions. *Pharm Biotechnol* 1995; **6:** 495–524.

31 Vogel FR. Improving vaccine performance with adjuvants. *Clin Infect Dis* 2000; **30** (suppl 3): 266–70.

32 Stanberry LR, Spruance SL, Cunningham AL, et al. Glycoprotein-D-adjuvant vaccine to prevent genital herpes. *N Engl J Med* 2002; **347:** 1652–61.

33 Galloway DA. Papillomavirus vaccines in clinical trials. *Lancet Infect Dis* 2003; **3:** 469–75.

34 Goldie SJ, Grima D, Kohli M, Wright TC, Weinstein M, Franco E. A comprehensive natural history model of HPV infection and cervical cancer to estimate the clinical impact of a prophylactic HPV-16/18 vaccine. *Int J Cancer* 10–10–2003; **106:** 896–904.

35 Jansen KU, Shaw AR. Human papillomavirus vaccines and prevention of cervical cancer. *Annu Rev Med* 2004; **55:** 319–31.

36 Mandic A, Vujkov T. Human papillomavirus vaccine as a new way of preventing cervical cancer: a dream or the future? *Ann Oncol* 2004; **15:** 197–200.

37 Munoz N, Bosch FX, Castellsague X, et al. Against which human papillomavirus types shall we vaccinate and screen? the international perspective. *Int J Cancer* 20–8-2004; **111:** 278–85.

38 Department of Health and Human Services, Centers for Disease Control and Prevention Division of STD Prevention. Prevention of Genital HPV Infection and Sequelae: Report of an External Consultants' Meeting. http://www.cdc.gov/nchstp/dstd/Reports_ Publications/99HPVReport.htm (accessed Oct 25, 2004).