Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial

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Summary

Background **A randomised double-blind placebo-controlled phase II study was done to assess the efficacy of a prophylactic quadrivalent vaccine targeting the human papillomavirus (HPV) types associated with 70% of cervical cancers (types 16 and 18) and with 90% of genital warts (types 6 and 11).**

Methods **277 young women (mean age 20·2 years [SD 1·7]) were randomly assigned to quadrivalent HPV (20 μg type 6, 40 μg type 11, 40 μg type 16, and 20 μg type 18) L1 virus-like-particle (VLP) vaccine and 275 (mean age 20·0 years [1·7]) to one of two placebo preparations at day 1, month 2, and month 6. For 36 months, participants underwent regular gynaecological examinations, cervicovaginal sampling for HPV DNA, testing for serum antibodies to HPV, and Pap testing. The primary endpoint was the combined incidence of infection with HPV 6, 11, 16, or 18, or cervical or external genital disease (ie, persistent HPV infection, HPV detection at the last recorded visit, cervical intraepithelial neoplasia, cervical cancer, or external genital lesions caused by the HPV types in the vaccine). Main analyses were done per protocol.**

Findings **Combined incidence of persistent infection or disease with HPV 6, 11, 16, or 18 fell by 90% (95% CI 71–97, $p<0·0001$) in those assigned vaccine compared with those assigned placebo.**

Interpretation **A vaccine targeting HPV types 6, 11, 16, 18 could substantially reduce the acquisition of infection and clinical disease caused by common HPV types.**

Introduction

Up to 70% of sexually active women will become infected with human papillomavirus (HPV) during their lifetime.[1] HPV infection causes about 470 000 cases of cervical cancer per year.[2] Although most cases of cervical cancer arise in the developing world where organised screening programmes with the Pap test have not been implemented, about 35 000 women die from this disease every year in the USA and Europe.[2] Even though screening reduces the risk of cervical cancer, it does not prevent HPV infection or development of precancerous lesions,[3] which need careful follow-up and often need excision.[4] Moreover, HPV infections that manifest as genital warts arise in 1–2% of young adults,[5] for which treatment is expensive and painful, and recurrences are common.[6] A diagnosis of genital warts might also cause sexual dysfunction and emotional disruption.[7]

More than 35 types of HPV infect the genital tract.[8] Of these, types 16 and 18 cause about 70% of cervical cancer and high-grade cervical intraepithelial neoplasia (CIN);[1] HPV 6 and 11 cause 90% of anogenital warts.[6] A prophylactic vaccine that targets these types should thus substantially reduce the burden of HPV-associated clinical diseases.

HPV is a non-enveloped, encapsulated, double-stranded DNA virus.[9] Expression of the L1 protein in heterologous systems (eg, yeast cells) generates non-infectious virus-like-particles (VLP) that resemble HPV virions.[9] In a placebo-controlled study,[10] a yeast-produced HPV 16 L1 VLP vaccine was 100% efficacious in prevention of CIN caused by HPV 16 infection 17 months after vaccination in women who were HPV 16 naive at the time of vaccination, and results from studies[11–15] have also shown HPV 11 or 18 L1 VLP vaccines to be highly immunogenic. These trials thus served as the basis for assessment of a quadrivalent HPV vaccine that targets HPV 6, 11, 16, and 18.

Methods

Study design

A phase II randomised, multicentre, double-blind placebo-controlled study of a quadrivalent HPV (type 6, 11, 16, and 18) L1 VLP vaccine was done in two parts. Part A was a sequential dose-escalation safety assessment, in which participants, investigators, and staff were blinded as to assignment of vaccine or placebo, but not to assignment of doses in the active-treatment group. Part B was a fully blinded dose-ranging assessment of

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immunogenicity and efficacy. Study procedures for individuals in part A and part B were identical. The results presented in this article are from part B.

1158 women aged 16–23 years were recruited in Brazil, Europe, and the USA. The study enrolled healthy women, who were not pregnant, had no previous abnormal Pap smears, and reported a lifetime history of four or fewer male sex partners. Enrolment of virgins was restricted to women who were 18 years or older and who were seeking contraception. This study did not exclude women with previous HPV infection. Participants were required to use effective contraception during the trial.

The active quadrivalent vaccine was a mixture of four recombinant HPV type-specific VLPs (Merck Research Laboratories, West Point, PA, USA) consisting of the L1 major capsid proteins of HPV 6, 11, 16, and 18 synthesised in *Saccharomyces cerevisiae*.[10,14,16] The four VLP types were purified and adsorbed onto amorphous aluminium hydroxyphosphate sulfate adjuvant. The placebo consisted of the same adjuvant and was visually indistinguishable from vaccine.

Three preparations of a quadrivalent HPV types 6, 11, 16, and 18 L1 VLP were used. The three preparations were: 20 μg type 6, 40 μg type 11, 40 μg type 16, and 20 μg type 18, with 225 μg aluminium adjuvant; 40 μg type 6, 40 μg type 11, 40 μg type 16, and 40 μg type 18, with 225 μg aluminium adjuvant; and 80 μg type 6, 80 μg type 11, 40 μg type 16, and 80 μg type 18, with 395 μg aluminium adjuvant. The study had two placebo groups with adjuvant doses of 225 μg or 450 μg for appropriate safety comparisons.

0·5 mL vaccine or placebo was given by intramuscular injection at day 1, month 2, and month 6. After vaccination, participants were observed for 30 min. Temperatures were also recorded orally every day in the evening for 5 days after vaccination, and the participant noted adverse events by standard diary card for 14 days after vaccination.

Gynaecological examination was done at day 1 and at months 7, 12, 24, and 36. A ThinPrep™ Pap test (Cytyc, Boxborough, MA, USA) and external genital, lateral vaginal, and cervical swabs for PCR analysis of HPV were obtained from all participants at day 1 and at months 7, 12, 18, 24, 30, and 36. Biopsy samples of external genital lesions identified during the study were taken, and serum samples were obtained at day 1 and months 2, 3, 6, 7, 12, 18, 24, 30, and 36.

This study was done in accordance with national or local requirements for ethics-committee review, informed consent, and other statutes or regulations regarding the protection of the rights and welfare of those participating in biomedical research. All individuals, or their parents or legal guardians, gave written informed consent after review of the protocol procedures.

The aim of the study was to assess a quadrivalent HPV L1 VLP vaccine in terms of the composite primary

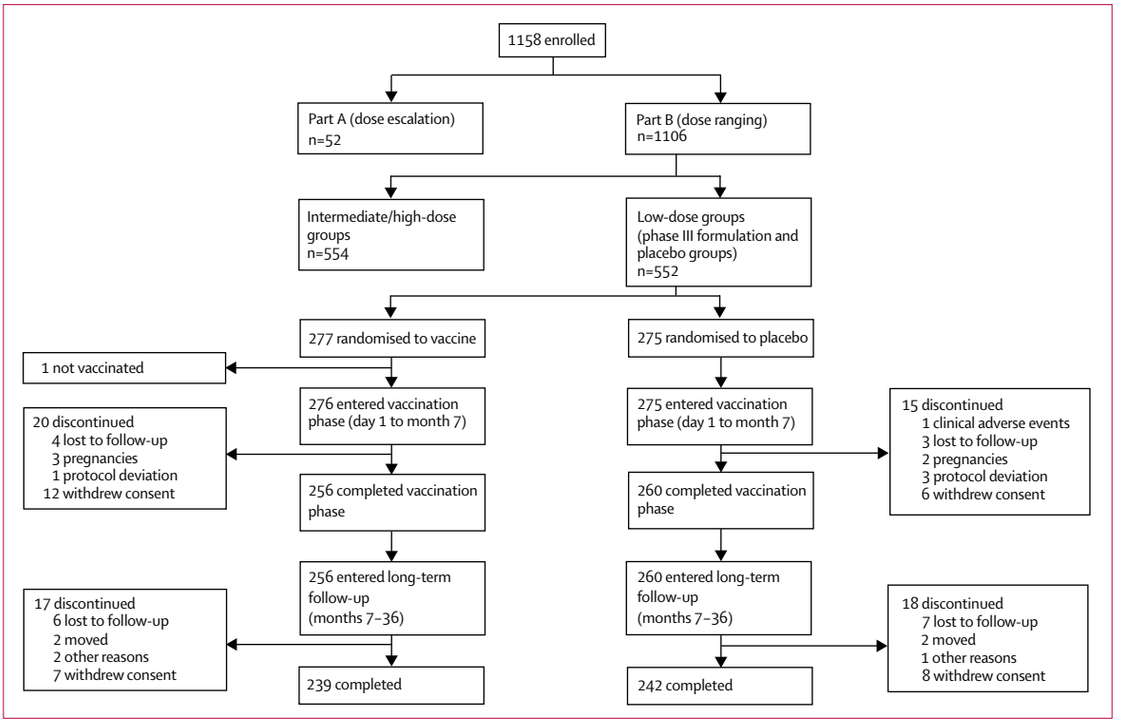

Figure 1: **Trial profile**

endpoint of persistent infection associated with HPV 6, 11, 16, or 18, or cervical or external genital disease compared with placebo. Women with persistent infection were defined as those who had the same vaccine-HPV-type DNA in cervicovaginal samples obtained 7 months after vaccination as those obtained from two or more consecutive visits (required to be 4 months or longer apart unless at least one tissue sample was diagnosed as cervical disease by a panel of pathologists), or as those who had vaccine-HPV-type DNA detected in a sample recorded during the last visit before being lost to follow-up. HPV-associated disease was defined as a tissue sample diagnosed as CIN by a panel of pathologists 7 months after vaccination; vulval intraepithelial neoplasia; vaginal intraepithelial neoplasia; external genital warts; or cervical, vulval, or vaginal cancer with vaccine-HPV-type DNA detected in tissue from, or in a swab of, the same lesion and in cervicovaginal samples obtained at the visit before the biopsy visit.

Laboratory analyses

All Pap tests and histological assessment were done in the setting of the study. Pap tests were reported in accordance with the Bethesda 2001 System.[17] Women underwent colposcopy if they were diagnosed with atypical squamous cells, in which high-grade squamous epithelial lesions could not be excluded; low-grade squamous intraepithelial lesions; high-grade squamous intraepithelial lesions; or atypical glandular cells. After the release of the American Society for Colposcopy and Cervical Pathology Biopsy Guidelines for management of atypical squamous cells of undetermined importance,[18] the protocol was modified so that residual liquid from these Pap tests was analysed by Hybrid Capture II™ (Digene, Gaithersburg, MD, USA) testing. Patients with a positive result on either low-risk or high-risk HPV probes underwent colposcopy.

All participants had colposcopy at the end of the study. At colposcopy, biopsy samples of discrete abnormalities were taken with separate instruments and were processed separately for histopathological analysis to avoid HPV contamination. A sample of the same lesion or a sample adjacent to the biopsied lesion was submitted for HPV typing. Biopsy samples were processed and read by a central laboratory (Diagnostic Cytology Laboratories, Indianapolis, IN, USA) for medical management. Endpoint assignment was done by use of consensus diagnoses from a panel of pathologists (RJK, MHS, BMR, and AF) who were blinded to central-laboratory diagnoses, treatment group, and HPV status.[19]

	Vaccine (n=277)	Placebo (n=275)	Population to which category applies		
			Per-protocol efficacy	Per-protocol immunogenicity	Modified intention to treat efficacy
Received ⩾1 injection*	276	275			
Excluded from per-protocol efficacy analyses					
HPV 6 or 11	59	61			
Positive to HPV 6 or 11†	28	39	•	•	•
Other‡	31	22			
HPV 16	75	72			
Positive to HPV 16†	49	51	•	•	•
Other‡	26	21			
HPV 18	49	46			
Positive to HPV 18†	17	21	•	•	•
Other‡	32	25			
Other reason for exclusion§					
General protocol violation	32	25			
Vaccine temperature out of range	6	6	•	•	
Enrolled in another study	0	1	•	•	
Incomplete vaccination series	17	13	•	•	
Incorrect clinical material or dose amount	1	0	•	•	
Received non-study vaccine	5	0	•	•	
Cervical ablation before day 1	3	0	•		
Month 7 swab out of acceptable day range	1	6	•		
Sexual intercourse within 48 h of visit at day 1 or month 7	0	1	•		
Second or third vaccination out of acceptable day range	12	14		•	
Missing month 7 serology sample or results	2	3		•	
Month 7 serum sample out of acceptable day range	4	15		•	
Excluded from modified intention-to-treat cohort¶					
HPV 6 or 11 (positive to HPV 6 or 11)	25	28			
HPV 16 (positive to HPV 16)	44	42			
HPV 18 (positive to HPV 18)	14	16			

*Participants may be included in more than one cohort. †Seropositive at day 1, PCR positive to the relevant HPV type at or before month 7, or both. ‡See other reason for exclusion. §Cumulated for all subtypes in per-protocol efficacy analysis. Women were counted only once in each category, and may appear in more than one category. ¶Seropositive or PCR positive, or both, for relevant HPV type at day 1.

Table 1: **Summary of participants excluded from analysis**

Swabs, biopsy samples and, later, thin tissue sections cut adjacent to sections used for histopathological analysis were used to detect HPV DNA with primers specific for HPV 6, 11, 16, or 18. Serum concentrations of antibodies to HPV 6, 11, 16, and 18 were measured with a competitive immunoassay (Luminex Corporation, Austin, TX, USA).[20] Antibody titres were determined in a competitive format—ie, known, type-specific phycoerythrin-labelled, neutralising antibodies[21,22] compete with serum antibodies from the participant for binding to conformationally sensitive, neutralising epitopes on VLPs.

To define the serostatus cutoff (ie, the lowest level of the assay's quantifiable range that can be reliably distinguished from negative samples), positivity rates for about 500 samples were assessed at 11 cutoffs ranging from 8 MU/L to 48 MU/L in increments of 4 MU/L. Before testing, serum samples were classified into panels according to their potential for being a true positive on the basis of clinical history and PCR results. Serostatus cutoff was then selected as the lowest titre so that all, or nearly all, known PCR-negative samples and likely negative samples yielded negative results. Dilution-corrected serostatus cutoffs were 20 MU/L for HPV type 6, 16 MU/L for type 11, 20 MU/L for type 16, and 24 MU/L for type 18. The dilution-corrected limits of detection were 4·1 MU/L for type 6, 3·0 MU/L for type 11, 10·2 MU/L for type 16, and 2·9 MU/L for type 18.

	Vaccine (n=277)	Placebo (n=275)
Age (years)		
Mean (SD)	20·2 (1·7)	20·0 (1·7)
Ethnic origin		
Asian	7 (3%)	11 (4%)
Black	25 (9%)	18 (7%)
Hispanic	14 (5%)	20 (7%)
White	216 (78%)	214 (78%)
Other	15 (5%)	12 (4%)
Country of origin		
USA	125 (45%)	126 (46%)
Brazil	94 (34%)	93 (34%)
Europe	58 (21%)	56 (20%)
Age at first sexual intercourse (years)		
Mean (SD)	16·7 (1·8)	16·7 (1·8)
Lifetime number of sexual partners		
0	17 (6%)	16 (6%)
1	80 (29%)	88 (32%)
2	73 (26%)	75 (27%)
3	67 (24%)	50 (18%)
4	40 (14%)	46 (17%)
Median (range)	2 (0–4)	2 (0–4)
Pap test		
Without Pap test at day 1	10 (4%)	6 (2%)
Unsatisfactory	3 (1%)	2 (>1%)
Negative for SIL	231 (83%)	238 (87%)
SIL Present	33 (12%)	29 (11%)
Atypical squamous cells of undetermined importance	16 (6%)	17 (6%)
Low-grade SIL	15 (5%)	10 (4%)
High-grade SIL	1 (>1%)	2 (>1%)
Atypical glandular cells	1 (>1%)	0
Contraceptive use*		
Male condom	63 (23%)	76 (28%)
Behaviour†	48 (17%)	48 (17%)
Hormonal	161 (58%)	157 (57%)
Other	21 (8%)	17 (6%)

SIL=squamous intraepthelial lesion. Data are number (%) unless otherwise stated. *The same participant may appear in more than one category. †Abstinence, withdrawal, or rhythm method.

Table 2: **Characteristics by vaccination group at enrolment**

Statistical analysis

Randomisation schedules were computer generated by use of a blocking factor of eight. Subjects were allocated in a 2:2:2:1:1 ratio to: HPV type 6, 11, 16, and 18 at doses of 20, 40, 40, and 20 μg, respectively, doses of 40, 40, 40, 40 μg, respectively, or doses of 80, 80, 40, 80 μg, respectively of quadrivalent L1 VLP vaccine; or to placebo with 225 μg or 450 μg of amorphous aluminium hydroxyphosphate sulfate adjuvant, respectively.

Primary efficacy analyses were done in the HPV 6/11, 16, and 18 per-protocol efficacy cohorts, which consisted of women who were naive for the relevant HPV type at enrolment, remained free of infection with the same vaccine HPV type through completion of the vaccination regimen, had all three doses of vaccine or placebo, and did not violate the protocol. Efficacy cases were counted starting after month 7.

To test the efficacy of the 20, 40, 40, 20 μg dose against persistent infection or disease associated with HPV 6, 11, 16, or 18, a one-sided test of the null hypothesis that the vaccine efficacy was 0 versus the hypothesis that vaccine efficacy was more than 0 at the $\alpha=0\cdot025$ level was done; p values were not adjusted for multiple hypothesis testing. Thus, rejection of the null hypothesis required the lower bound of the two-sided 95% CI for vaccine efficacy to exceed 0%. An exact conditional procedure was used to assess vaccine efficacy with the assumption that the numbers of cases in the vaccine and placebo groups are independent Poisson random variables.[23] Individual follow-up was calculated as the number of person-years between the specified starting time and the final visit date, the date the participant became a case (ie, developed an endpoint), or the date the participant underwent definitive treatment (cervical endpoints only). If a woman developed more than one endpoint, her date of becoming a case was the date when the first endpoint was detected.

Overall, 20 women with the composite endpoint of persistent infection or diseases associated with the vaccine HPV types were needed for the study to have 89·8% power to declare the vaccine efficacious with a two-sided $\alpha=0\cdot05$, assuming a true vaccine efficacy of 80%. Therefore, enrolment of about 250 participants in the placebo group and in the low-dose vaccine group was needed.

Secondary analyses were done for a modified intention-to-treat population that included all participants who were naive (ie, seronegative and PCR negative) to the relevant HPV type at enrolment and who had had at least one vaccination. Efficacy cases in this population were counted from day 30. Immunogenicity was measured in a per-

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	Vaccine (n=276)				Placebo (n=275)				Efficacy difference (95% CI)	p
	n*	Events	Women-years at risk	Incidence per 100 women-years at risk	n*	Events	Women-years at risk	Incidence per 100 women-years at risk		
Outcome†										
Infection or disease associated with HPV 6, 11, 16, or 18	235	4	566·8	0·7	233	36	536·5	6·7	90 (71–97)	<0·0001
Infection associated with HPV 6, 11, 16, or 18	235	4	566·5	0·7	233	35	536·9	6·5	89 (70–97)	<0·0001
Disease associated with HPV 6, 11, 16, or 18	235	0	568·8	0	233	6	563·0	1·1	100 (16–100)	0·0151
External genital lesion‡	235	0	566·9	0	233	3	561·1	0·5	NA	NA
CIN	235	0	562·2	0	233	3	552·5	0·5	NA	NA
Outcome by HPV type										
HPV 6 associated	214	0	517·5	0	209	13	501·2	2·6	100 (68–100)	<0·0001
HPV 11 associated	214	0	517·5	0	209	3	503·7	0·6	NA	NA
HPV 16 associated	199	3	484·4	0·6	198	21	465·4	4·5	86 (54–97)	<0·0001
HPV 18 associated	224	1	541·8	0·2	224	9	536·9	1·7	89 (21–100)	0·0103

NA=Number of events too small for meaningful efficacy estimates. *Number who had at least one follow-up visit. †A participant arises only once within each category, but may be in more than one category. ‡Defined as condylomata acuminata, vulvar intraepithelial neoplasia, or vaginal intraepithelial neoplasia.

Table 3: **Efficacy of quadrivalent vaccine against persistent infection or disease associated with HPV 6, 11, 16, or 18 in per-protocol population**

protocol immunogenicity cohort, defined as members of the per-protocol efficacy cohort who were vaccinated and who had had serum samples obtained during the protocol-specified time frames, irrespective of HPV infection or disease status after month 7. This study presents the efficacy, immunogenicity, and tolerability of this low-dose vaccine compared with the pooled placebo groups. This low-dose vaccine was chosen for phase III studies.

Role of the funding source

Sponsor staff and clinical-site investigators designed the study. Sponsor staff were responsible for field monitoring, data entry, data review (including integrity and consistency checks), testing of clinical samples for HPV DNA and HPV immune responses, and analysis of data. All authors provided input into interpretation of the data, and writing and revising of the report. The corresponding author had full access to the data and had final responsibility for the decision to submit for publication.

Results

277 women were randomly assigned to quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP vaccine (20, 40, 40, 20 μg, respectively) and 275 to placebo (figure 1). 431 (78%) were included in the per-protocol efficacy analyses for HPV types 6/11, 404 (73%) for type 16, and 456 (83%) for type 18 (table 1, figure 1). 275 (99%) women assigned low-dose vaccine and 275 (100%) assigned placebo were included in the safety analyses. The main reasons for exclusion from the per-protocol cohort were seropositivity to a vaccine HPV type at day 1 or presence of HPV 6, 11, 16, or 18 DNA before completion of the vaccination regimen. The HPV 6/11, 16, and 18 modified intention-to-treat cohorts included 498, 465, and 521 women, respectively.

Table 2 shows participants' baseline characteristics of the HPV vaccine and placebo cohorts. Characteristics of the modified intention-to-treat and per-protocol efficacy populations were much the same as those of the overall study cohort (data not shown).

The main efficacy analyses were done in the per-protocol efficacy cohort (table 3). Over the 30 months' follow-up after vaccination, combined incidence of persistent HPV 6, 11, 16, or 18 infection or associated genital disease decreased by 90% (95% CI 71–97) in women assigned vaccine compared with those assigned placebo (table 3). Of the 40 primary HPV endpoint cases, 13 were from detection of HPV 6, 11, 16, or 18 DNA on samples obtained at the last visit of record (three in the vaccination group [all at month 36] and ten in the placebo group [eight of ten at month 36]). HPV 16 DNA was detected in three women in the vaccine group at the last recorded visit, and one woman had verified, persistent HPV 18 infection detected at months 12 and 18 only. In the modified intention-to-treat cohort, the efficacy of the vaccine with regard to the primary endpoint was 89% (95% CI 73–96, $p<0·0001$; table 4).

Findings from interim immunogenicity analysis showed the lowest dose vaccine induced serological antibody responses to HPV 6, 11, 16, or 18 that were much the same as those of the intermediate-dose and high-dose preparations.[24] The combined incidence of persistent infection with HPV 6, 11, 16, or 18 or associated genital disease did not differ between the high-dose, intermediate-dose, or low-dose groups (incidence per 100 women-years at risk 0·7, 1·3, and 0·5, respectively). Vaccine efficacy with regard to the composite endpoint of infection or disease associated with HPV 6, 11, 16, or 18 for the three doses pooled (n=701) was 88% (95% CI 76–94).

	Vaccine (n=276)				Placebo (n=275)				Efficacy difference (95% CI)	p
	n*	Events	Women-years at risk	Incidence per 100 women-years at risk	n*	Events	Women-years at risk	Incidence per 100 women-years at risk		
Infection or disease associated with HPV 6, 11, 16, or 18†	266	6	723·6	0·8	263	48	667·1	7·2	89 (73–96)	<0·0001
Infection associated with HPV 6, 11, 16, or 18†	256	6	720·5	0·8	254	47	665·6	7·1	88 (72–96)	<0·0001
Disease associated with HPV 6, 11, 16, or 18†	266	0	728·5	0	263	10	712·4	1·4	100 (56–100)	0·0009
External genital lesion‡	265	0	725·9	0	261	4	712·4	0·6	NA	NA
CIN	258	0	716·9	0	256	7	700·7	1·0	100 (32–100)	0·0072

NA=number of events too small for meaningful efficacy estimates. *Number who had at least one follow-up visit. †A participant arises only once in each category, but may be in more than one category. ‡Defined as condylomata acuminata, vulvar intraepithelial neoplasia, or vaginal intraepithelial neoplasia.

Table 4: **Efficacy of quadrivalent HPV vaccine against persistent infection or disease associated with HPV 6, 11, 16, or 18 in modified intention-to-treat population**

Vaccine-induced immune responses were assessed in the HPV 6 and 11, HPV 16, and HPV 18 per-protocol immunogenicity cohorts, which included 208, 194, and 219 women assigned vaccine, respectively. Concentrations of antibodies to HPV associated with protection from infection have not been defined.[20] The immune response to the vaccine was observationally compared with the serum antibody concentrations to HPV in those assigned placebo who had been infected with vaccine HPV types, had subsequently mounted an immune response, and had apparently cleared infection before enrolment (ie, who were seropositive and PCR negative for the relevant HPV type at enrolment). In those assigned placebo, geometric mean titre of antibodies to HPV remained constant throughout the 3 years of the study.

All women assigned active vaccine in the per-protocol immunogenicity cohorts developed detectable antibody responses to HPV 6, 11, 16, and 18 at completion of the vaccine regimen (ie, month 7). Vaccine-induced geometric mean titres of antibodies were substantially higher in women assigned active vaccine than in those assigned placebo who had a previous history of natural HPV infection (table 5). Although mean antibody titres in those assigned quadrivalent HPV L1 VLP vaccine started to decline after month 7 (data not shown), at month 36 they remained at or above the titres recorded for women who had an immune response, presumably associated with clearance of HPV infection. Of women who had a valid immunoassay result at month 36, 173 (94%) of 184 were seropositive for HPV type 6, 176 (96%) of 184 for type 11, all 177 for type 16, and 149 (76%) of 196 for type 18. Furthermore, antibody titres for 174 (89%) of the 196 women positive for HPV 18 were above the assay's lower limit of quantification. Analyses of antibody titres in four women assigned quadrivalent vaccine who developed persistent HPV 18 infection or who were detected with HPV 16 DNA at one visit before loss to follow-up were non-informative because they developed robust antibody responses at month 7. However, antibody titres to HPV 18 after month 7 for the participant who had persistent infection with HPV 18 were slightly lower than for most of those who were allocated active vaccine.

The low-dose HPV vaccine was generally well tolerated (table 6). Adverse events at the injection site were higher in women allocated active vaccine than in those allocated placebo. Pain was the most common injection-site adverse event and headache the most common systemic adverse event. Most (94%) adverse events were of mild or moderate intensity. Only one patient (in the placebo group) discontinued treatment, because of hypoaesthesia thought not to be caused by the placebo. There were no vaccine-related serious adverse events. Neither intermediate-dose nor the high-dose quadrivalent HPV vaccine was excluded from assessment in phase III trials on the basis of an unacceptable safety profile. However, a modest increase (from 3% to 6%) in injection-site adverse events was recorded with the higher doses (data not shown).

	Month 7				Month 36			
	Vaccine (n=276)		Placebo (n=275)*		Vaccine (n=276)		Placebo (n=275)*	
	n	GMT (MU/L, 95% CI)	n	GMT (MU/L, 95% CI)	n	GMT (MU/L, 95% CI)	n	GMT (MU/L, 95% CI)
HPV 6	208	582 (527–643)	17	55 (28–108)	184	93 (81–108)	16	68 (33–139)
HPV 11	208	697 (618–785)	4	94 (5–1639)	184	94 (81–110)	4	96 (19–498)
HPV 16	194	3892 (3324–4558)	15	37 (17–85)	177	509 (436–593)	15	29 (12–69)
HPV 18	219	801 (694–925)	12	42 (23–75)	196	60 (49–74)	10	29 (15–59)

GMT=geometric mean titre. *Baseline seropostive and PCR negative for specific HPV type.

Table 5: **Geometric mean titres of antibodies to HPV 6, 11, 16, and 18 by vaccination group in per-protocol immunogenicity population**

	Vaccine (n=272)*	Placebo (n=274)†
Adverse events reported	250 (92%)	242 (88%)
Injection site	234 (86%)	212 (77%)
Systemic	187 (69%)	190 (69%)
Vaccine-associated adverse events	243 (89%)	225 (82%)
Injection site	234 (86%)	212 (77%)
Systemic	104 (38%)	90 (33%)
Serious adverse events	2 (1%)	2 (1%)

Data are number (%). *Excludes three women without follow-up and one who had incorrect material or dose. †Excludes one woman without safety follow-up.

Table 6: **Clinical adverse events**

Discussion

We have shown that a multivalent vaccine is efficacious against HPV types that cause cancer and genital warts. Over 35 months' follow-up, incidence of persistent infection associated with HPV 6, 11, 16, or 18 decreased by 89% in women allocated active vaccine who had at least one dose (ie, the modified intention-to-treat population) compared with those allocated placebo. Vaccine efficacy was 90% in the per-protocol efficacy population, suggesting that the vaccine was protective even during the vaccination period. For example, during the course of vaccination (day 1 through month 7), three women assigned active vaccine and five women assigned placebo were detected with HPV 18 DNA. Of these, only one was verifiable persistent infection (in the placebo group). Thus, one woman allocated placebo and no women allocated active vaccine developed persistent HPV 18 infection during the vaccination period. Furthermore, efficacy with regard to clinical disease associated with HPV 6, 11, 16, or 18 was 100%.

This study was not originally powered to assess vaccine efficacy for the disease endpoints or for each HPV type separately. However, the fact that all three women with external genital lesions and all three with CIN were in the placebo group is encouraging in terms of protection against these disease endpoints. Analysis of the incidence of the main efficacy endpoint in the two higher-dose groups supported the decision to select the low dose of quadrivalent HPV L1 VLP vaccine as the final dose for assessment in the phase III programme.

The quadrivalent HPV vaccine was highly immunogenic. All women allocated active vaccine developed higher detectable antibody responses to HPV at month 7 than did those allocated placebo who had natural infection and who remained at higher or similar antibody titres throughout the study. Because women are at risk of HPV infection for as long as they are sexually active, protection induced by a HPV vaccine must be long-lived. At month 36, more than 94% of women were seropositive for HPV 6, 11, and 16; fewer (76%) women had antibody responses against HPV 18 at month 36, although 89% had antibody titres for HPV 18 above the assay's lower limit of quantification. Longer follow-up studies will be needed to assess the duration of efficacy of this quadrivalent HPV vaccine, and to determine whether booster doses will be needed.

Results from a previous study[10] found that a prototype yeast-produced HPV 16 L1 VLP vaccine given to women who were HPV 16-negative prevented acquisition of HPV 16 infection and associated CIN.[12] A bivalent HPV 16/18 vaccine prepared with AS04 adjuvant was efficacious in preventing persistent infections and associated cervical abnormalities over a 27-month period.[25]

In the developed world, full implementation of cervical-cancer screening has substantially shifted the burden of HPV infection from cervical-cancer mortality to management of precancerous lesions. In these countries, in addition to further reduction in the incidence of cervical cancer, universal HPV vaccination might decrease the medical, psychological, and economic costs associated with management of abnormalities detected by Pap testing and CIN. Inclusion of HPV 6 and 11 in a vaccine could diminish the incidence of genital warts and low-grade CIN that are prognostically benign, costly,[26] and disruptive psychologically.[7] In developing countries that have not implemented screening programmes for cervical cancer, the effect of a drop in rates of cervical cancer after implementation of universal HPV vaccination could be substantial. However, such implementation will need definition of the vaccine's effect on public health, cost-effectiveness, the optimum age for vaccination, and the duration of protection.

Universal HPV vaccination might be most effective when implemented in 10–13 year olds, who are likely to be HPV negative.[27–29] The expectation that the vaccine will reduce cervical-cancer rates, the fact that HPV infection affects most women, and the lack of an effective means to prevent HPV infection in sexually active people lend support to the vaccination of preadolescents. The benefit of vaccination against oncogenic HPV types 16 and 18 is likely to be negligible in heterosexual men because they rarely develop HPV-associated genital cancers. However, a vaccine against the non-oncogenic HPV types 6 and 11 could be considered in the prophylaxis of genital warts in men and in women.

This study has shown that a candidate HPV 6, 11, 16, and 18 vaccine was generally well tolerated, induced high-titres of serum antibodies to HPV types, and effectively prevented acquisition of infection and clinical disease caused by common HPV types. Large-scale studies are under way.

Contributors

E Barr and G M Tamms developed the study protocol based on a previous study designed by L A Koutsky and K U Jansen with input and support of K A Ault, C M Wheeler, D R Brown, A R Giuliano, D M Harper, F E Skjeldestad, M Lehtinen, and L L Villa. K U Jansen oversaw the Merck HPV vaccine research and development programme. E Barr, A J Saah, and G M Tamms managed headquarters operations at Merck Research Laboratories. L L Villa, R L R Costa, C A Petta, R P Andrade, M Lehtinen, C Malm, F E Skjeldestad, S-E Olsson, M Steinwall, K A Ault, D R Brown, C M Wheeler, A R Giuliano, D M Harper, and L A Koutsky set up the study sites. K U Jansen and F J Taddeo developed the PCR-based HPV 6, 11, 16, and 18 detection assays and tested cervicovaginal samples using

the assays. K U Jansen and M T Esser developed the antiHPV 6, 11, 16, and 18 immunoassays and tested study serum samples. R J Kurman, M H Stoler, B M Ronnett, and A Ferenczy reviewed the histology slides. J Yu, L Lupinacci, and R Railkar developed and implemented the data-analysis plan.

Main co-investigators

Brazil—C Goes, G Andreoni, R Carneiro, E Fukazawa, J Mesquita, F Coelho, and M Perrotti.
Finland—R Heikkila and R Zilliacus.
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Sweden—A Andersson-Ellstrom, K Elfgren, and G von Krogh.
USA—J T Comerci, R P Edwards, S A Gall, C M Peterson, and Y C Wade.

Conflict of interest

E Barr, M T Esser, K U Jansen, F J Taddeo, A J Saah, J Yu, L Lupinacci, R Railkar, H L Sings, and G M Tamms are employees of Merck Research Laboratories, a division of Merck & Co, and potentially own stock and hold stock options in the company, or both. Merck is developing the quadrivalent HPV vaccine and funded this clinical trial. K A Ault, D R Brown, D M Harper, R J Kurman, F E Skjeldestad, and L L Villa have received honoraria from Merck Research Laboratories given for consultation work or membership in the Phase III HPV Vaccine Steering and Registries Oversight Committees, or both, during the past 2 years. A Ferenczy, R J Kurman, B M Ronnett, and M H Stoler are members of the HPV Vaccine Program Pathology Panel. As such, they have been paid for developing the Panel's standard operating procedures and for histopathological readings of biopsy slides. R P Andrade, K A Ault, D R Brown, R L R Costa, A R Giuliano, L A Koutsky, R J Kurman, M Lehtinen, C Malm, C A Petta, F E Skjeldestad, D M Harper, and C M Wheeler led clinical sites that participated in the study and were compensated for all activities related to execution of the study. They are also receiving similar funding for their work on Merck's HPV vaccine phase III programme. L L Villa and A Ferenczy have been given honoraria for lectureships on behalf of Merck's HPV vaccine programme. D R Brown, A R Giuliano, L L Villa, and C M Wheeler have been paid for consultations regarding the HPV vaccine programme in men. K A Ault is a member of Merck's HPV Vaccine Obstetrics and Gynecology Advisory Board and as such receives honoraria for consultative work. D M Harper is a member of Merck's HPV Vaccine Young Adult Primary Care Advisory Board. F E Skjeldestad has received funding from Merck Research Laboratories in support of a natural history study of HPV disease in young Norwegian women.

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References

1 Bosch FX, de Sanjose S. Chapter 1: Human papillomavirus and cervical cancer-burden and assessment of causality. *J Natl Cancer Inst Monogr* 2003; **31:** 3–13.
2 Ferlay J, Bray F, Pisani P. GLOBOCAN 2002: cancer incidence, mortality and prevalence worldwide. IARC CancerBase No 5. Version 2.0. Lyon: IARC Press, 2004.
3 Peto J, Gilham C, Fletcher O, Matthews FE. The cervical cancer epidemic that screening has prevented in the UK. *Lancet* 2004; **364:** 249–56.
4 Sadler L, Saftlas A, Wang W, et al. Treatment for cervical intraepithelial neoplasia and risk of preterm delivery. *JAMA* 2004; **291:** 2100–06.
5 Persson G, Andersson K, Krantz I. Symptomatic genital papillomavirus infection in a community: incidence and clinical picture. *Acta Obstet Gynecol Scand* 1996; **75:** 287–90.
6 von Krogh G. Management of anogenital warts (condylomata acuminata). *Eur J Dermatol* 2001; **11:** 598–604.
7 Maw RD, Reitano M, Roy M. An international survey of patients with genital warts: perceptions regarding treatment and impact on lifestyle. *Int J STD AIDS* 1998; **9:** 571–78.
8 de Villiers E-M, Fauquet C, Broker TR, et al. Classification of papillomaviruses. *Virology* 2004; **324:** 17–27.
9 Zhou J, Sun XY, Stenzel DJ, Frazer IH. Expression of vaccinia recombinant HPV 16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles. *Virology* 1991; **185:** 251–57.
10 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.
11 Emeny RT, Wheeler CM, Jansen KU, et al. Priming of human papillomavirus type 11-specific humoral and cellular immune responses in college-aged women with a virus-like particle vaccine. *J Virol* 2002; **76:** 7832–42.
12 Brown DR, Fife KH, Wheeler CM, et al. Early assessment of the efficacy of a human papillomavirus type 16 L1 virus-like particle vaccine. *Vaccine* 2004; **22:** 2936–42.
13 Fife KH, Wheeler CM, Koutsky LA, et al. Dose-ranging studies of the safety and immunogenicity of human papillomavirus type 11 and type 16 virus-like particle candidate vaccines in young healthy women. *Vaccine* 2004; **22:** 2943–52.
14 Ault K, Giuliano AR, Edwards R, et al. A phase I study to evaluate a human papillomavirus (HPV) type 18 L1 VLP vaccine. *Vaccine* 2004; **22:** 3004–07.
15 Brown DR, Bryan JT, Schroeder JM, et al. Neutralization of human papillomavirus type 11 (HPV-11) by serum from women vaccinated with yeast-derived HPV-11 L1 virus-like particles: correlation with competitive radioimmunoassay titer. *J Infect Dis* 2001; **184:** 1183–86.
16 Lowe RS, Brown DR, Bryan JT, et al. Human papillomavirus type 11 (HPV-11) neutralizing antibodies in the serum and genital mucosal secretions of African green monkeys immunized with HPV-11 virus-like particles expressed in yeast. *J Infect Dis* 1997; **176:** 1141–45.
17 Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda system: terminology for reporting results of cervical cytology. *JAMA* 2002; **287:** 2114–19.
18 Wright TC Jr, Cox JT, Massad LS, et al. 2001 consensus guidelines for the management of women with cervical cytological abnormalities. *JAMA* 2002; **287:** 2120–29.
19 Stoler MH, Schiffman M. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL triage study. *JAMA* 2004; **285:** 1500–06.
20 Opalka D, Lachman CE, MacMullen SA, et al. Simultaneous quantitation of antibodies to neutralizing epitopes on virus-like particles for human papillomavirus types 6, 11, 16, and 18 by a multiplexed luminex assay. *Clin Diagn Lab Immunol* 2003; **10:** 108–15.
21 Christensen ND, Reed CA, Cladel NM, et al. Monoclonal antibodies to HPV-6 L1 virus-like particles identify conformational and linear neutralizing epitopes on HPV-11 in addition to type-specific epitopes on HPV-6. *Virology* 1996; **224:** 477–86.
22 Christensen ND, Dillner J, Eklund C, et al. Surface conformational and linear epitopes on HPV-16 and HPV-18 L1 virus-like particles as defined by monoclonal antibodies. *Virology* 1996; **223:** 174–84.
23 Chan ISF, Bohidar NR. Exact power and sample size for vaccine efficacy studies. *Theory Meth* 1998; **27:** 1305–22.
24 Villa L, Costa R, Petta C, et al. A dose-ranging safety and immunogenicity study of a quadrivalent HPV (types 6/11/16/18) L1 VLP vaccine in women. HPV 20th International Papillomavirus Conference, Paris, France, Oct 4–9, 2002.
25 Harper DM, Franco EL, Wheeler CM, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection of human papillomavirus type 16 and 18 in young women: a randomised controlled trial. *Lancet* 2004; **364:** 1757–65.
26 Insinga RP, Glass AG, Rush BB. The health care costs of cervical human papillomavirus-related disease. *Am J Obstet Gynecol* 2004; **191:** 114–20.
27 Grunbaum JA, Kann L, Kinchen S, et al. Youth risk behavior surveillance—United States, 2003. *MMWR Surveill Summ* 2004; **53:** 1–96.
28 Wellings K, Nanchahal K, Macdowall W, et al. Sexual behavior in Britain: early heterosexual experience. *Lancet* 2001; **358:** 1843–50.
29 Johnson AM, Mercer CH, Erens B, et al. Sexual behavior in Britain: partnerships, practices, and HIV risk behaviors. *Lancet* 2001; **358:** 1835–42.