Efficacy of a quadrivalent prophylactic human papillomavirus (types 6, 11, 16, and 18) L1 virus-like-particle vaccine against high-grade vulval and vaginal lesions: a combined analysis of three randomised clinical trials

Elmar A Joura, Sepp Leodolter, Mauricio Hernandez-Avila, Cosette M Wheeler, Gonzalo Perez, Laura A Koutsky, Suzanne M Garland, Diane M Harper, Grace W K Tang, Daron G Ferris, Marc Steben, Ronald W Jones, Janine Bryan, Frank J Taddeo, Oliver M Bautista, Mark T Esser, Heather L Sings, Micki Nelson, John W Boslego, Carlos Sattler, Eliav Barr, Jorma Paavonen

Summary

Background Vulval and vaginal cancers among younger women are often related to infection with human papillomavirus (HPV). These cancers are preceded by high-grade vulval intraepithelial neoplasia (VIN2–3) and vaginal intraepithelial neoplasia (VaIN2–3). Our aim was to do a combined analysis of three randomised clinical trials to assess the effect of a prophylactic quadrivalent HPV vaccine on the incidence of these diseases.

Methods 18 174 women (16–26 years) were enrolled and randomised to receive either quadrivalent HPV6/11/16/18 L1 virus-like-particle vaccine or placebo at day 1, and months 2 and 6. Individuals underwent detailed anogenital examination at day 1, 1 month after dose three, and at 6–12-month intervals for up to 48 months. Suspect genital lesions were biopsied and read by a panel of pathologists and vaccine HPV type-specific DNA testing was done. The primary endpoint was the combined incidence of VIN2–3 or VaIN2–3 associated with HPV16 or HPV18. Primary efficacy analyses were done in a per-protocol population.

Findings The mean follow-up time was 3 years. Among women naive to HPV16 or HPV18 through 1 month after dose three (per-protocol population; vaccine n=7811; placebo n=7785), the vaccine was 100% effective (95% CI 72–100) against VIN2–3 or VaIN2–3 associated with HPV16 or HPV18. In the intention-to-treat population (which included 18 174 women who, at day 1, could have been infected with HPV16 or HPV18), vaccine efficacy against VIN2–3 or VaIN2–3 associated with HPV16 or HPV18 was 71% (37–88). The vaccine was 49% (18–69) effective against all VIN2–3 or VaIN2–3, irrespective of whether or not HPV DNA was detected in the lesion. The most common treatment-related adverse event was injection-site pain.

Interpretation Prophylactic administration of quadrivalent HPV vaccine was effective in preventing high-grade vulval and vaginal lesions associated with HPV16 or HPV18 infection in women who were naive to these types before vaccination. With time, such vaccination could result in reduced rates of HPV-related vulval and vaginal cancers.

Lancet 2007; 369: 1693–702

Department of Gynecology and Obstetrics, Medical University of Vienna, Vienna, Austria (E A Joura MD, Prof S Leodolter MD); **National Institute of Public Health, Cuernavaca, Morelos, Mexico** (Prof M Hernandez-Avila MD); **Departments of Molecular Genetics and Microbiology and Obstetrics and Gynecology, University of New Mexico, Albuquerque, NM, USA** (Prof C M Wheeler PhD); **National Research Center, Group Saludcoop, Bogotá, Colombia** (G Perez MD); **Department of Epidemiology, University of Washington, Seattle WA, USA** (Prof L A Koutsky PhD); **Microbiology and Infectious Diseases Department, Royal Women's Hospital and Department of Obstetrics and Gynecology, University of Melbourne, Melbourne, Victoria, Australia** (Prof S M Garland MD); **Norris Cotton Cancer Center, Departments of Obstetrics and Gynecology and Community and Family Medicine, Dartmouth Medical School, Hanover NH, USA** (Prof D M Harper MD); **Department of Obstetrics and Gynecology, University of Hong Kong, Hong Kong, SAR, China** (Prof G W K Tang MD); **Department of Family Medicine and Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA, USA** (Prof D G Ferris MD); **Direction Risques Biologiques, Environnementaux et Occupationnels, Institut National de Santé Publique du Québec, Montréal, Québec, Canada** (Prof M Steben MD); **Vulvar Clinic, Department of Gynecologic Oncology, National Women's Hospital,**

Introduction

The female genital tract, a continuum of squamous epithelium from the vulva to the cervix, is commonly infected by human papillomavirus (HPV). The outcome of HPV infection depends on the viral genotype (low risk or high risk/carcinogenic) and the site of infection (the cervical squamocolumnar junction is more susceptible to HPV disease). Carcinogenic HPV can cause cervical, anal, vulval, and vaginal cancers.[1–6] Compared with cervical cancer, vulval and vaginal cancers develop less frequently. In the UK, vulval cancer is six times and vaginal cancer twenty times less common than cervical cancer.[7] Nonetheless, vulval and vaginal cancer collectively account for about 6% of all gynaecological cancers. By contrast with secondary cancer prevention programmes for breast and cervical cancers, no screening programmes exist for vaginal and vulval malignancies.

As with cervical intraepithelial neoplasia grade 2–3, high-grade vulval and vaginal lesions—ie, vulval intraepithelial neoplasia grade 2–3 (VIN2–3) and vaginal intraepithelial neoplasia grade 2–3 (VaIN2–3)—are precursors to HPV-related invasive cancers of these areas.[5,6] Although the true incidence of vaginal intraepithelial neoplasia is unknown, the incidence of vulval carcinoma in situ (vulval intraepithelial neoplasia grade 3) increased more than 400% in the USA between 1973 and 2000; invasive vulval cancer increased by 20% during the same period.[8] The rate of vulval carcinoma in situ has also been increasing worldwide and seems to be associated with HPV infection, especially with HPV16 and HPV18.[9–11]

The annual progression rate of untreated vulval carcinoma in situ to invasive cancer is at least 10%; by contrast, cervical intraepithelial neoplasia grade 3 progresses at a rate of about 2%.[12] Patients with vaginal intraepithelial neoplasia have a 2% risk of developing invasive cancer.[13] Treatment of vulval and vaginal intraepithelial neoplasia is challenging, can be disfiguring, and requires very long-term follow-up, since disease recurrence is common.[14–16] Together, these data suggest that, as with cervical

Auckland, New Zealand (R W Jones MD)**; Merck Research Laboratories, West Point, PA, USA** (J Bryan PhD, F J Taddeo PhD, O M Bautista PhD, M T Esser PhD, H L Sings PhD, M Nelson BS, J W Boslego MD, C Sattler MD, E Barr MD)**; and Department of Obstetrics and Gynecology, University Central Hospital, Helsinki, Finland** (Prof J Paavonen MD)

Correspondence to: Prof Jorma Paavonen, Department of Obstetric and Gynecology, University Central Hospital, Helsinki, Finland Haartmaninkatu 2, 00290 Helsinki Finland **jorma.paavonen@hus.fi**

intraepithelial neoplasia grade 2–3, high-grade vulval and vaginal lesions are surrogate markers for potential development of HPV-related vulval or vaginal cancer.

Prophylactic administration of a quadrivalent HPV6/11/16/18 L1 virus-like-particle (VLP) vaccine has been shown to be 99% efficacious against cervical intraepithelial neoplasia grade 2–3 or adenocarcinoma in situ associated with HPV16 or HPV18 infection.[17] The vaccine was also highly effective against disease caused by infection with HPV6 and HPV11, which are the cause of most anogenital warts and a proportion of low-grade neoplasias.[18–20] We did a combined analysis of three randomised clinical trials of this vaccine to assess its effect on the rates of high-grade vulval and vaginal lesions associated with HPV16 and HPV18, as well as its effect on overall rates of such lesions, irrespective of whether or not HPV DNA was detected in the lesion.

	Protocol 007	Protocol 013	Protocol 015
General			
Phase	IIb	III	III
Trial registry number	NCT00365716	NCT00092521	NCT00092534
Number enrolled	552	5455	12 167
Study dates	2000 to 2004	2001 to 2007*	2002 to 2007*
Study sites	International, multicentre	International, multicentre	International, multicentre
Blinding	Double-blind	Double-blind	Double-blind
Study vaccine	Quadrivalent vaccine or placebo	Quadrivalent vaccine or placebo	Quadrivalent vaccine or placebo
Vaccination regimen (months)	0, 2, and 6	0, 2, and 6	0, 2, and 6
Visit schedule (months)	0, 7, 12, 18, 24, 30, and 36	0, 3, 7, 12, 18, 24, 30, 36, and 48	0, 7, 12, 24, 36, and 48
Inclusion and exclusion criteria			
Age (years)	16–23	16–23	16–26
Lifetime number of sexual partners	0–4	0–4	0–4
Previous abnormal pap smear	Not allowed (but no exclusion if no prior cytology available)		
Previous HPV-related disease	Not allowed (but no exclusion on basis of current/ongoing infection or evidence of CIN, VIN, or VaIN). Individuals with visible genital warts were not enrolled		
Comorbidities, co-treatments	Few restrictions: history of severe allergic reaction; known immune disorders; receipt of attenuated or live vaccines within 14–21 days of enrolment		
Average length of follow-up after dose three (years)	2·5	2·5	2·5
Colposcopy and biopsy			
Requirement for biopsy	All lesions that in the opinion of the investigator were possibly, probably, or definitely HPV-related, or whose diagnosis could not be ascertained		
Laboratory processing	DCL Laboratory, Indianapolis, IN, USA		
Pathology interpretation (routine)	DCL Laboratory, Indianapolis, IN, USA (used for medical management)		
Pathology diagnosis (endpoints)	Blinded pathology panel (used for endpoint determination)		
HPV causality assessment	PCR assay on paraffin-embedded specimens (Merck Research Laboratories)		

CIN=cervical intraepithelial neoplasia. VaIN=vaginal intraepithelial neoplasia. VIN=vulval intraepithelial neoplasia. *Protocols 013 and 015 were ongoing at the time of this report. The prespecified cutoff dates for this combined analysis were June 15, 2006 (protocol 013) and June 16, 2006 (protocol 015).

Table 1: **Study design and comparison of protocols 007, 013, and 015**

Methods

Patients and procedures

18 174 women aged 16–26 years were enrolled in one of three double-blind placebo-controlled randomised trials.[18,21,22] Participants were drawn from 157 sites in 24 countries in the Americas, Europe, and Asia. Most study sites were located in university settings and in urban clinics. In Finland, enrolment was population based. In Latin America, enrolment was based in neighbourhood health centres in many cases. Table 1 compares the design features of the three individual studies. Non-pregnant, healthy women without report of a previous abnormal pap smear and with a lifetime history of four or fewer sexual partners were eligible for enrolment. Participants were asked to use effective contraception during the vaccination period. To reduce potential interference with the immunological response and reactogenicity of the study vaccine or placebo, the protocols prohibited the administration of non-study inactivated or recombinant vaccines (eg, tetanus, influenza, or meningococcal vaccine) 14 days before or after any dose of study vaccine. Individuals were not enrolled if they were concurrently taking part in clinical studies of investigational agents or studies involving the collection of cervical specimens.

At enrolment, women underwent a comprehensive anogenital examination that included visual inspection of the perianal area, vulva, and vagina with the naked eye or magnifying glass, or if warranted, with a colposcope (low magnification). Individuals also underwent pap cervical cytology screening (ThinPrep, Cytyc Corporation, Marlborough, MA, USA), plus collection of cervical and anogenital swabs for HPV DNA testing.

Individuals were randomly assigned to receive either quadrivalent HPV6/11/16/18 L1 VLP vaccine (Merck and Co, Whitehouse Station, NJ, USA) or placebo, both containing 225 µg of Merck's amorphous aluminium hydroxyphosphate sulfate adjuvant. Study vaccine or placebo was administered at day 1, month 2, and month 6. In protocol 007, randomisation schedules were computer-generated with a blocking factor of eight. Individuals were randomised in a 2:2:2:1:1 ratio to receive either vaccine containing 20 µg HPV6 L1 VLP, 40 µg HPV11 L1 VLP, 40 µg HPV16 L1 VLP, and 20 µg HPV18 L1 VLP; 40 µg HPV6 L1 VLP, 40 µg HPV11 L1 VLP, 40 µg HPV16 L1 VLP, and 40 µg HPV18 L1 VLP; 80 µg HPV6 L1 VLP, 80 µg HPV11 L1 VLP, 40 µg HPV16 L1 VLP, and 80 µg HPV18 L1 VLP; placebo with 225 µg adjuvant; or placebo with 450 µg adjuvant. In this analysis, we included only those individuals from protocol 007 who received the low-dose formulation and the pooled placebo arms.

In protocols 013 and 015, participants were randomised in a 1:1 ratio within each study centre to receive quadrivalent vaccine (20 µg HPV6 L1 VLP, 40 µg HPV11 L1 VLP, 40 µg HPV16 L1 VLP, and 20 µg HPV18 L1 VLP) or placebo, by use of a computer-generated randomised

allocation schedule and an interactive voice response system. Embedded within protocol 013 were two substudies designed to address immunogenicity hypotheses unrelated to the study's primary efficacy analysis. Every individual enrolled in protocol 013 was enrolled in one of these substudies (protocols 011 and 012). In protocol 011, 466 individuals received quadrivalent vaccine and hepatitis B vaccine and 467 individuals received placebo and hepatitis B vaccine. Protocol 012 was an immunogenicity bridging study of the monovalent HPV16 vaccine component and the quadrivalent vaccine. Individuals in protocol 012 who received monovalent HPV16 vaccine were not included in our efficacy analyses. No individual was enrolled in both substudy protocols. Overall randomisation in protocol 013 was controlled via the randomisation into the two substudies with permuted block sizes of four and 13 for protocols 011 and 012, respectively. In protocol 015, individuals were allocated to treatment assignment using permuted blocks of size six.

Individuals returned to the study site for genital examination and collection of cervical and anogenital swabs at follow-up visits scheduled 1 and 6 months after dose three, and 6–12 months thereafter. The anogenital swab was defined as a sweep of the labial, vulvar, and perineal region with a dacron swab. A second dacron swab was used for the perianal region. These two swabs were placed in the same container of specimen transport medium. The endo/ectocervical swab was collected with a dacron swab of the opening of the cervix and ectocervix from anterior to posterior cervical lip. All external anogenital lesions that, in the opinion of the investigator, were possibly, probably, or definitely HPV related, or whose diagnosis could not be ascertained, were to be biopsied. If several lesions were present, the investigator was requested to obtain at least one specimen from each area affected (vagina, periurethral area, left labia, right labia, perineum, perianal area). Within a given area, if lesions of more than one morphology were present (eg, flat *vs* exophytic, pigmented *vs* skin-coloured), the investigator was instructed to biopsy a representative sample of each morphology.

Infection with HPV6/11/16/18 was determined with PCR and serology. Cervical and anogenital swabs collected between day 1 and month 7, inclusively, were tested for HPV6/11/16/18 DNA with PCR-based assays.[18,21–24] Biopsy samples were processed and read for clinical management at a central laboratory (Diagnostic Cytology Laboratories, Indianapolis, IN, USA) and then read for endpoint determination by a panel of four pathologists who were blinded to central laboratory and clinical diagnoses, vaccination group, and HPV status. Sections adjacent to those used for histological assessment were sent to the sponsor's central PCR laboratory and HPV type-specific assays were done as described previously.[18]

In all studies, serum samples collected at enrolment were tested for presence of neutralising type-specific anti-HPV antibodies.[25] An audit done by the sponsor concluded that there was a deviation from the standard operating procedure for testing a subset of serum samples from the combined studies, including about 5·5% of day 1 serology results. All day 1 sera that were tested out of compliance with the standard operating procedure were retested.

A case was defined as a pathology panel consensus diagnosis of VIN2–3 or VaIN2–3, or invasive vulval or vaginal cancer, with HPV16 or HPV18 DNA detected in an adjacent section from the same tissue block (phase III), adjacent biopsy tissue (phase IIb), or a biopsy swab (defined as a dacron swab that was wiped over the biopsy site; phase IIb). In the phase IIb study, if HPV16 or HPV18 DNA was detected in an adjacent biopsy tissue or a biopsy swab specimen, then at least one cervicovaginal swab specimen obtained from the individual at the visit immediately before the biopsy had to be positive for the same HPV type. For analyses of all cases of VIN2–3 and VaIN2–3, irrespective of causal HPV type or irrespective of whether or not HPV DNA was detected in the lesion, a case was defined as one of the above endpoints.

The studies conformed with applicable country or local requirements with regard to ethical committee review, informed consent, and the protection of the rights and welfare of human individuals participating in biomedical research.

Statistical analysis

Vaccine efficacy was defined as (1–relative risk)×100%, where the relative risk is the ratio of the incidence rate in the vaccine group over the incidence rate in the placebo group. Corresponding 95% CI were estimated with an exact conditional procedure that assumed that the number of cases in the vaccine and placebo groups were independent Poisson random variables.[26] 95% CI (not adjusted for multiple testing) that do not include zero indicate a nominally significant difference at the $\alpha=0\cdot05$ (two-sided) level. Integrated estimates of vaccine efficacy against high-grade vulval and vaginal intraepithelial lesions were computed by pooling individuals across the studies by vaccination group. Homogeneity of vaccine efficacy across the three studies was informally assessed before data from the three studies were combined to obtain integrated estimates of vaccine efficacy. Although protocols 007, 013, and 015 are similar in many ways, there are small differences among them (table 1). In protocol 013, there was an additional study visit at month 3. In protocol 007, colposcopies and biopsies were done on the basis of voluntary pap triage guidelines, whereas protocols 013 and 015 had mandatory pap triage guidelines. Protocol 015 required pap screening every 12 months, whereas protocols 007 and 013 required pap screening every 6 months. Furthermore, the three studies enrolled individuals from different regions of the world. For the study endpoints presented here, study-specific estimates of vaccine efficacy and the

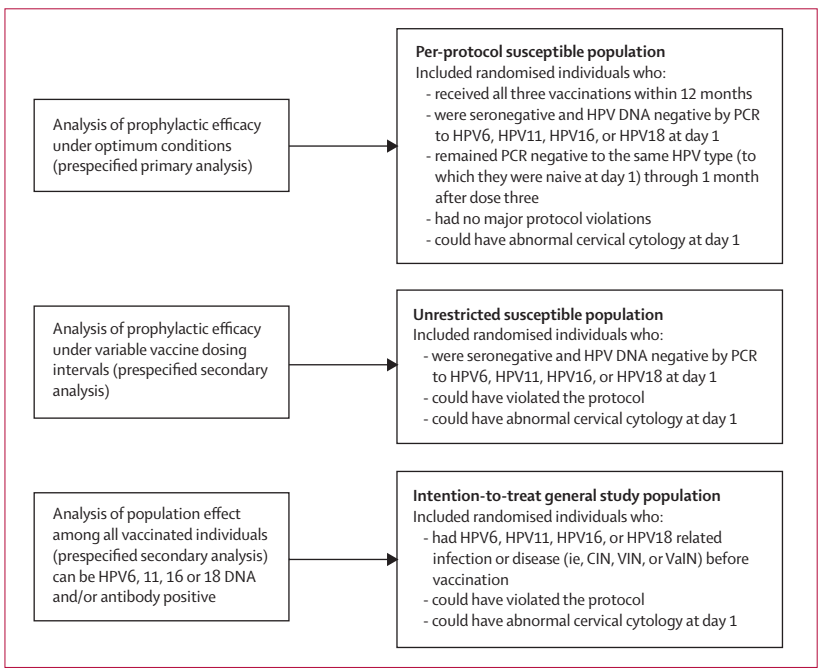

Figure 1: **Populations for efficacy analyses**

corresponding 95% CI were computed for the purpose of informally assessing the consistency, across studies, of the overall estimates of vaccine efficacy. Here, analyses were done with respect to the primary composite endpoint of HPV16-related or HPV18-related high-grade vulval or vaginal intraepithelial lesions (ie, VIN2–3 or VaIN2–3 associated with HPV16 or HPV18) and with respect to any VIN2–3 or VaIN2–3, irrespective of whether or not HPV DNA was associated with the lesion. Analyses were also done by lesion type and by HPV type. A woman with a single lesion containing both HPV16 and HPV18 DNA, or with both a VIN2–3 and a VaIN2–3 lesion, counted only once towards the primary composite endpoint and once for each of the component endpoints defined on the basis of HPV type or lesion type. If an individual developed more than one endpoint, she was counted as an endpoint-case at the date of the first endpoint. No formal tests of hypotheses were done for any of the endpoints reported here. We did not adjust CI for vaccine efficacy for multiplicity.

The primary analysis of efficacy was done in the per-protocol population, defined as individuals who were HPV16 or HPV18 DNA negative by PCR and seronegative to HPV16 or HPV18 at enrolment, who remained PCR negative to the same HPV type to which they were naive at enrolment through 1 month after dose three (also known as month 7), who received three doses of vaccine or placebo within 1 year, and who did not violate the protocol in ways that could interfere with the assessment of vaccine efficacy (figure 1). Abnormal cervical cytology at enrolment was not an exclusion criterion for the per-protocol population. The per-protocol population models a population in which immunisation is completed before exposure to HPV16 or HPV18. Follow-up for endpoint ascertainment in the per-protocol population started 1 month after dose three.

To estimate vaccine efficacy in a less restrictive setting, prespecified analyses were done in an unrestricted susceptible population, which included all individuals who were seronegative and PCR-negative to HPV16 or HPV18 at day 1 (figure 1). Follow-up for endpoint ascertainment in this population started 1 day after the first dose of vaccine or placebo. We also estimated vaccine efficacy in an intention-to-treat population of individuals, irrespective of baseline HPV status or evidence of HPV-related anogenital disease (figure 1). Follow-up for endpoint ascertainment in this population started after

	Overall		Protocol 007 (n=552)	Protocol 013 (n=5455)	Protocol 015 (n=12 167)
	Vaccine (n=9087)	Placebo (n=9087)			
Age (years)	20·0 (2·0)	20·0 (2·0)	20·1 (1·7)	20·3 (1·8)	19·9 (2·1)
Region					
Asia-Pacific	349/9087 (4%)	353/9087 (4%)	..	521/5455 (10%)	181/12 167 (1%)
North America	1381/9087 (15%)	1383/9087 (15%)	251/552 (45%)	1597/5455 (29%)	916/12 167 (8%)
Latin America	2800/9087 (31%)	2795/9087 (31%)	187/552 (34%)	2215/5455 (41%)	3193/12 167 (26%)
Europe	4557/9087 (50%)	4556/9087 (50%)	114/552 (21%)	1122/5455 (21%)	7877/12 167 (65%)
Age at first sexual intercourse (years)*	16·7 (1·9)	16·7 (1·9)	16·7 (1·8)	16·9 (1·9)	16·6 (1·9)
Median lifetime number of sex partners*	2	2	2	2	2
Past pregnancy	2057/9083 (23%)	2041/9084 (22%)	131/552 (24%)	1505/5452 (28%)	2460/12 164 (20%)
Cervical cytological abnormality	1011/8694 (12%)	988/8658 (11%)	62/531 (12%)	604/5400 (11%)	1351/11 815 (11%)
Positive to HPV6/11/16, or 18 by PCR	1329/8983 (15%)	1317/8996 (15%)	64/550 (12%)	743/5398 (14%)	1839/12 031 (15%)
Positive to HPV6/11/16, or 18 by serology	1792/9067 (20%)	1788/9066 (20%)	101/551 (18%)	1067/5441 (20%)	2412/12 141 (20%)

Data are number of individuals with indicated characteristic/number of individuals assessable for the indicated characteristic (%) or mean (SD). *Computed statistics are among individuals who were not virgins at enrolment.

Table 2: **Selected baseline characteristics of the study population at enrolment**

day 1. In this population, for each vaccination group, the non-parametric product-limit estimate of the cumulative incidence distribution was computed with the Kaplan-Meier method and presented graphically for the composite endpoint of VIN2–3 or VaIN2–3 associated with HPV16 or HPV18 and also for any VIN2–3 or VaIN2–3.[27] This analysis reflects study information gathered through a mean follow-up time of 3 years (range 0–6 years; 25th to 75th percentile: 2·9–3·2 years).

Role of the funding source

The trials were designed, managed, and analysed by the study sponsor in conjunction with external investigators plus a data and safety monitoring board. The sponsor collated the data, monitored the conduct of the study, did the statistical analysis, and participated in the writing of the manuscript with all authors. The authors were actively involved in the collection, analysis, or interpretation of the data, the revising of the manuscript for intellectual content, and approved the final manuscript. All authors had full access to all the data. EAJ and JP had final responsibility for the decision to submit for publication.

Results

Of the 18 174 women enrolled, 18 150 received at least one injection of quadrivalent vaccine or placebo. Baseline characteristics were much the same in the two randomised groups (table 2). The composition of the populations included in the efficacy analyses is shown in table 3.

In the per-protocol susceptible population, 15 women developed histologically confirmed VIN2–3 or VaIN2–3 that was associated with HPV16 or HPV18; all cases were in women who received placebo (vaccine efficacy 100%, 95% CI 72–100; table 4). In the unrestricted susceptible population, there were an extra 14 cases in those who received placebo and one case in a woman who received the vaccine compared with the per-protocol susceptible population; vaccine efficacy was 97% (95% CI 79–100; table 4). Of the placebo cases, 27 were HPV16 related and three were HPV18 related (table 4). The single case in the vaccine group was VIN2–3 associated with HPV16.

Compared with the unrestricted susceptible population, the intention-to-treat population included an extra eight cases of VIN2–3 or VaIN2–3 associated with HPV16 or HPV18 in those who received vaccine and an extra two cases in those who received placebo; for all these extra cases, the individuals were infected with HPV16 or HPV18 before vaccination. In the intention-to-treat population, the quadrivalent vaccine reduced the incidence of VIN2–3 or VaIN2–3 associated with HPV16 or HPV18 by 71% (95% CI 37–88; table 4). Five cases, all in the placebo group, were associated with HPV6 rather than with type HPV16 or HPV18. None was associated with HPV11.

When all cases of high-grade lesions were assessed in the intention-to-treat population, irrespective of whether or not HPV DNA was detected in the lesion and irrespective of causal HPV type, we found 27 cases in those who received vaccine and 53 cases in those who received placebo. Vaccine efficacy was 49% (95% CI 18–69; table 4).

The benefit of vaccination with this vaccine in the intention-to-treat population was initially masked by prevalent infection or disease, against which the vaccine has little effect (figure 2). However, as vaccination prevented new HPV16 and HPV18 infections over the course of follow-up, reductions in the incidence of VIN2–3 or VaIN2–3 associated with HPV16 or HPV18 became more apparent.

In the intention-to-treat population, a minimally invasive perineal lesion (about 4×4 mm) was diagnosed in one 22-year-old individual, 18 months after receiving three doses of quadrivalent HPV vaccine.[18] The biopsy (about 2×2×1 mm) of this lesion was reported as a well differentiated squamous cell carcinoma by the study pathology panel. PCR of the biopsy was negative for

	Vaccine (n=9087)	Placebo (n=9087)	Analysis population to which exclusion category applies	
			Per-protocol susceptible	Unrestricted susceptible
Patients eligible for analysis				
Per-protocol susceptible population				
Analysis of HPV16/18-related VIN2/3 or VaIN2/3	7811	7785		
Analysis of HPV16-related VIN2/3 or VaIN2/3	6687	6500		
Analysis of HPV18-related VIN2/3 or VaIN2/3	7450	7381		
Unrestricted susceptible population				
Analysis of HPV16/18-related VIN2/3 or VaIN2/3	8757	8774		
Analysis of HPV16-related VIN2/3 or VaIN2/3	7530	7534		
Analysis of HPV18-related VIN2/3 or VaIN2/3	8383	8410		
Intention-to-treat general study population	9087	9087		
Reasons for exclusion*				
General protocol violations†	583	570	✓	
Missing day 1 swab samples/results	176	142	✓	✓
Day 1 swab sample out of acceptable day range	3	3	✓	✓
Day 1 serology sample out of acceptable day range	12	5	✓	✓
Missing day 1 serology samples/results	9	9	✓	✓
Missing month 7 swab samples/results	232	202	✓	
Seropositive and/or PCR positive to HPV16				
At day 1‡	1433	1445	✓	✓
At or before month 7 inclusive‡	1530	1750	✓	
Seropositive and/or PCR positive to HPV18				
At day 1‡	574	572	✓	✓
At or before month 7 inclusive‡	653	774	✓	

*Women with more than one reason for exclusion were included in each applicable category. †The most common general protocol violations were: incomplete vaccination series (500 individuals) and received non-study vaccine (182 individuals). ‡Applies only to the analysis populations for the respective HPV type; day 1 includes seropositivity or PCR positivity; post-day 1 includes PCR positivity only.

Table 3: **Number of individuals included in the efficacy analyses and reasons for exclusion from the indicated efficacy analysis populations**

	Vaccine (n=9087)			Placebo (n=9087)			Efficacy (95% CI)
	Number in given population	Cases	Rate (cases per 100 person-years at risk)	Number in given population	Cases	Rate (cases per 100 person-years at risk)	
Per-protocol susceptible population*							
HPV16-related or HPV18-related VIN2/3 or VaIN2/3	7811	0	0·00	7785	15†	0·08	100% (72 to 100)
HPV16-related VIN2/3 or VaIN2/3	6687	0	0·00	6500	13	0·08	100% (68 to 100)
HPV18-related VIN2/3 or VaIN2/3	7450	0	0·00	7381	2	0·01	100% (–427 to 100)
By lesion type							
HPV16-related or HPV18-related VIN2/3	7811	0	0·00	7785	8	0·04	100% (42 to 100)
HPV16-related VIN2/3	6687	0	0·00	6500	7	0·04	100% (33 to 100)
HPV18-related VIN2/3	7450	0	0·00	7381	1	0·01	100% (<–999 to 100)
HPV16-related or HPV18-related VaIN2/3	7811	0	0·00	7785	7	0·04	100% (31 to 100)
HPV16-related VaIN2/3	6687	0	0·00	6500	6	0·04	100% (18 to 100)
HPV18-related VaIN2/3	7450	0	0·00	7381	1	0·01	100% (<–999 to 100)
Unrestricted susceptible population‡							
HPV16-related or HPV18-related VIN2/3 or VaIN2/3	8757	1§	0·00	8774	29§	0·11	97% (79 to 100)
HPV16-related VIN2/3 or VaIN2/3	7530	1	0·00	7534	27	0·12	96% (78 to 100)
HPV18-related VIN2/3 or VaIN2/3	8383	0	0·00	8410	3	0·01	100% (–143 to 100)
By lesion type							
HPV16-related or HPV18-related VIN2/3	8757	1	0·00	8774	20	0·08	95% (69 to 100)
HPV16-related VIN2/3	7530	1	0·00	7534	20	0·09	95% (69 to 100)
HPV18-related VIN2/3	8383	0	0·00	8410	1	0·00	100% (<–999 to 100)
HPV16-related or HPV18-related VaIN2/3	8757	0	0·00	8774	9	0·03	100% (49 to 100)
HPV16-related VaIN2/3	7530	0	0·00	7534	7	0·03	100% (31 to 100)
HPV18-related VaIN2/3	8383	0	0·00	8410	2	0·01	100% (–435 to 100)
Intention-to-treat population¶							
HPV16-related or HPV18-related VIN2/3 or VaIN2/3	9087	9‖	0·03	9087	31‖	0·12	71% (37 to 88)
HPV16-related VIN2/3 or VaIN2/3	9087	8	0·03	9087	29	0·11	72% (38 to 89)
HPV18-related VIN2/3 or VaIN2/3	9087	1	0·00	9087	3	0·01	67% (–316 to 99)
By lesion type							
HPV16-related or HPV18-related VIN2/3	9087	8	0·03	9087	21	0·08	62% (10 to 85)
HPV16-related VIN2/3	9087	8	0·03	9087	21	0·08	62% (10 to 85)
HPV18-related VIN2/3	9087	0	0·00	9087	1	0·00	100% (<–999 to 100)
HPV16-related or HPV18-related VaIN2/3	9087	2	0·01	9087	11	0·04	82% (17 to 98)
HPV16-related VaIN2/3	9087	1	0·00	9087	9	0·03	89% (20 to 100)
HPV18-related VaIN2/3	9087	1	0·00	9087	2	0·01	50% (–863 to 99)
All high-grade lesions, intention-to-treat population**							
All VIN2/3 or VaIN2/3	9087	27††	0·10	9087	53††	0·20	49% (18 to 69)
All VIN2/3	9087	16	0·06	9087	33	0·12	51% (9 to 75)
All VaIN2/3	9087	12	0·05	9087	21	0·08	43% (–22 to 74)

*Includes individuals who were HPV DNA negative by PCR and seronegative to the relevant vaccine-HPV-type at enrolment, remained PCR negative to the same vaccine-HPV-type through 1 month after dose three, received three doses of vaccine or placebo within 1 year, and did not violate the protocol. Among those in the per-protocol population, the following had at least one follow-up visit after dose three: vaccine=7771 *vs* placebo=7742 for the analysis of HPV16/18 endpoints; vaccine=6653 *vs* placebo=6465 for the analysis of HPV16 endpoints; and vaccine=7413 *vs* placebo=7341 for the analysis of HPV18 endpoints. †Of the 15 cases in the placebo group, seven were from protocol 013 and eight were in protocol 015. ‡Includes individuals who were naive to the relevant vaccine-HPV-type at enrolment. Among those in the unrestricted susceptible population, the following received at least one dose and had at least one follow-up visit after dose one: vaccine=8642 *vs* placebo=8669 for the analysis of HPV16/18 endpoints; vaccine=7444 *vs* placebo=7445 for the analysis of HPV16 endpoints; and vaccine=8273 *vs* placebo=8313 for the analysis of HPV18 endpoints. §Of the 29 cases in the placebo group, one was from protocol 007, ten were from protocol 013, and 18 were from protocol 015. The singe case in the vaccine group was from protocol 13. ¶Includes individuals with prevalent anogenital disease and infections due to any high-risk or low-risk HPV type prevaccination. Among those in the intention-to-treat population, 8955 vaccine and 8965 placebo individuals received at least one dose and had at least one follow-up visit after dose one. ‖For eight of the nine vaccine cases and for two of the 31 placebo cases, individuals were infected with HPV16 or HPV18 before receiving the first dose. Of the 31 cases in the placebo group, one was from protocol 007, 11 were from protocol 013, and 19 were from protocol 015. Of the nine cases in the vaccine group, five were from protocol 013 and four from protocol 015. **Includes individuals with prevalent anogenital disease and infections due to any high-risk or low-risk HPV type prevaccination. ††Of the 53 cases in the placebo group, one was from protocol 007, 23 were from protocol 013, and 29 were from protocol 015. Of the 27 cases in the vaccine group, 17 were from protocol 013 and ten from protocol 015.

Table 4: **Vaccine efficacy in preventing high-grade vulval and vaginal lesions associated with HPV16 or HPV18, and all high-grade vulval and vaginal lesions (irrespective of cause)**

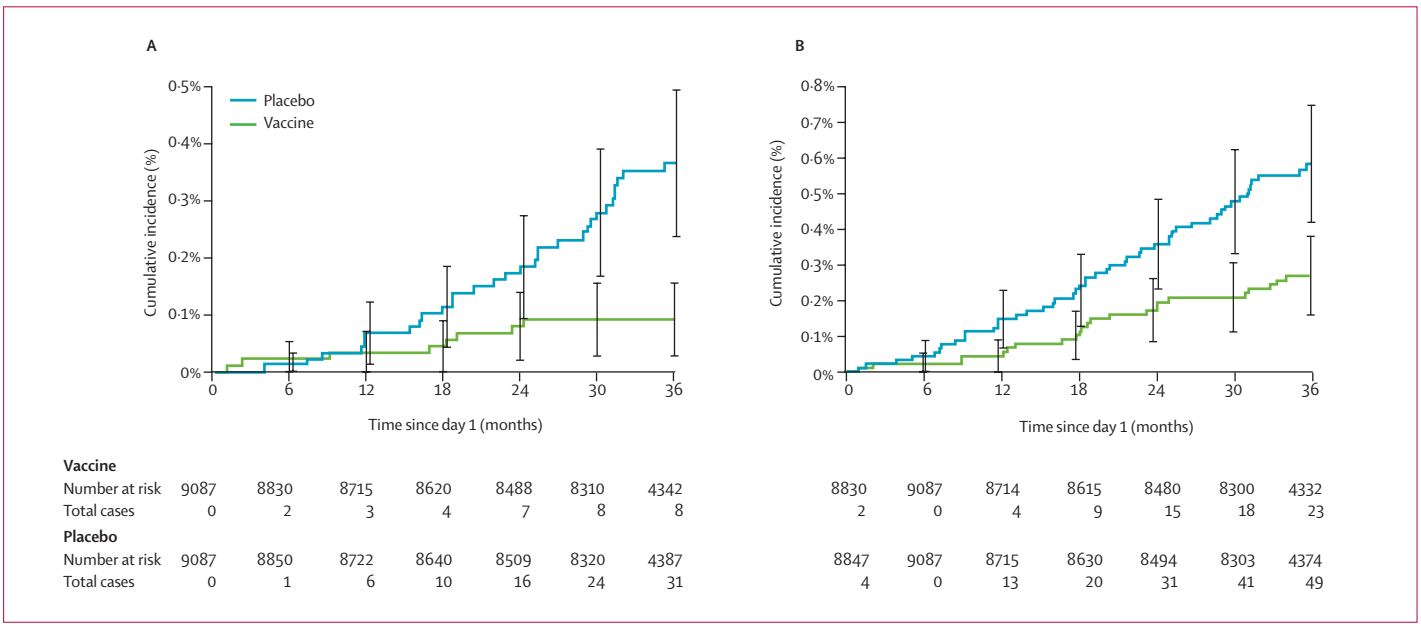

***Figure 2*: Time to event(s) in the intention-to-treat population**
Curves are displayed through 36 months because of the small number of individuals at risk after 36 months. (A) Time to HPV16-related or HPV18-related VIN2–3 or VaIN2–3. (B) Time to any VIN2–3 or VaIN2–3, irrespective of causal HPV type. Of the randomised individuals, 8955 vaccine and 8965 placebo individuals received at least one dose and had at least one follow-up visit after dose one.

vaccine-type HPV and for ten other oncogenic HPV types. Cervicovaginal swabs gathered during all scheduled visits (day 1, months 3, 7, 12, 18, 24, 30, and 36) were negative for HPV, except at the visit in which the perineal lesion was detected, when an external genital swab sample (of the labial-vulvar-perineal and perianal regions) was positive for HPV16 and HPV59. The perineal lesion was no longer visible 6 months later, and no further treatment of the lesion was provided. No further perineal lesions have been seen to date and the woman continues to be followed clinically for any residual or recurrent disease.

Across all efficacy populations, the incidence of VIN2–3 was higher than that of VaIN2–3, which is consistent with epidemiology data in the general population. During the study, in the placebo group of the intention-to-treat population, 64% (21/33) of the VIN2–3 cases were attributed to HPV16; five of the 29 cases of VIN2–3 or VaIN2–3 associated with HPV16 were also diagnosed (not necessarily concurrently) in women who were also diagnosed with HPV16-related cervical intraepithelial neoplasia grade 2–3 or adenocarcinoma in situ; none of the three cases of HPV18-related VIN2–3 or VaIN2–3 were diagnosed among women with HPV18-related cervical intraepithelial neoplasia grade 2–3 or adenocarcinoma in situ.

Safety was assessed in five clinical trials (protocols 007, 013, and 015, and two adolescent studies, protocols 016[28] and 018[29]), four of which were placebo controlled. In all except one of the clinical trials, safety was assessed with vaccination report card-aided surveillance for 14 days after each injection of quadrivalent vaccine or placebo. The female individuals who were monitored with such surveillance included 5088 women aged 9–26 years at enrolment who received quadrivalent vaccine, 3470 women aged 9–26 years at enrolment who received aluminium-containing placebo, and 320 girls aged 9–15 years at enrolment who received saline placebo. 13 (0·1%) individuals discontinued due to adverse experiences. The most common vaccine-related adverse reactions among female recipients of quadrivalent vaccine (frequency of at least 1% and greater than placebo) were fever (n=516 [10·3%] vaccine *vs* 320 [8·6%] placebo), nausea (211 [4·2%] vaccine *vs* 152 [4·1%] placebo), dizziness (142 [2·8%] vaccine *vs* 98 [2·6%] placebo), and injection-site pain (4203 [83·9%] vaccine *vs* 2572 [75·4%] aluminium-containing placebo), swelling (1271 [25·4%] vaccine *vs* 540 [15·8%] aluminium-containing placebo), erythema (1235 [24·6%] vaccine *vs* 628 [18·4%] aluminium-containing placebo), and pruritus (156 [3·1%] vaccine *vs* 97 [2·8%] aluminium-containing placebo). 206 of 21464 male and female individuals who received quadrivalent vaccine (n=11778) or placebo (n=9686) reported a serious systemic adverse experience. Of these, seven (0·06%) individuals reported a serious systemic adverse experience that was judged by the study investigator to be vaccine related. Rare cases (irrespective of causality) of bronchospasm (one individual) and asthma (two individuals) were reported as serious adverse events. Additionally, one individual had two serious injection-site adverse experiences (injection-site pain and injection-site joint movement impairment). Across the clinical studies, 18 deaths were reported in 21464 male and female individuals. The most common cause of

death was motor vehicle accident (four vaccine *vs* three placebo), followed by overdose or suicide (two vaccine *vs* two placebo), and pulmonary embolus or deep vein thrombosis (one vaccine *vs* one placebo). There were two cases of sepsis, one case of pancreatic cancer, and one case of arrhythmia in the group that received quadrivalent vaccine, and one case of asphyxia in the placebo group.

Discussion

Our study provides evidence that this prophylactic quadrivalent HPV vaccine, developed to prevent cervical cancer, also prevents HPV-related vulval and vaginal precancers in 16–26-year-old women. The vaccine was 97% effective in preventing VIN2–3 and VaIN2–3 associated with HPV16 or HPV18 in a population that was naive to these viruses at the time of first vaccination, and 100% effective in a population that was naive through completion of the vaccination regimen. Vaccine efficacy in the intention-to-treat population, which included women who could already have acquired HPV16 or HPV18 infection and those with vulval or vaginal HPV-related disease before vaccination,[30] was 71%, providing an estimate of the potential public-health benefit of an effective HPV vaccine. Additionally, a 49% reduction in all high-grade vulval and vaginal intraepithelial neoplasia, irrespective of whether or not HPV DNA was isolated from the lesion and irrespective of causal HPV type, was seen in the intention-to-treat population of sexually active young women.

The rarity of vulval and vaginal cancer in this age-group requires the use of VIN2–3 and VaIN2–3 as surrogate endpoints in HPV vaccine clinical trials. By combining data from three clinical trials that enrolled more than 18 500 women, we have provided the highest possible precision in the estimate of vaccine efficacy against VIN2–3 and VaIN2–3 associated with HPV16 and HPV18 available from clinical trials to date.

The HPV vaccine has already been shown to be 99% effective in preventing HPV16-related or HPV18-related cervical intraepithelial neoplasia grade 2–3 and adenocarcinoma in situ, both of which are obligate precursors of HPV16-related and HPV18-related invasive cervical cancer.[17] Previous studies have shown that vaccination is well tolerated.[18,21,22] The vaccine was approved after priority review by the US Food and Drug Administration on June 8, 2006. At the time of licensure (after about 2 years of follow-up) there was 100% efficacy against HPV6/11/16/18-related VIN2–3 (no cases in individuals who received vaccine *vs* eight cases in those who received placebo; 95% CI 41·4–100) and VaIN2–3 (no cases in those who received vaccine *vs* five cases in those who received placebo) in the per-protocol population. Our study includes an additional year of post-licensure follow-up.

We enrolled women from diverse settings, including low-income settings (eg, Lima, Sao Paulo, and Bogota) and areas of higher income (eg, Montreal, Glasgow, Helsinki, Copenhagen, Seattle, and Vienna). Although these clinical trials included a broad representation of women from developed and developing countries, 16–26-year-old women with a median number of more than two lifetime sexual partners were under-represented, as were women with a history of abnormal pap tests or genital warts. Although the exact effect of these exclusions cannot be determined, they presumably affect both the numbers of HPV-susceptible women and the numbers of women with prevalent HPV infections entering the trials. This bias could have been balanced, in part, by including women who had no previous pap test; however, the exact number of such women is unknown. Because of these exclusion criteria, it is possible that the observed efficacy in the intention-to-treat population is overestimated. From this perspective, this low-risk population of enrolled women might not be fully comparable to the general population. Additionally, women with HIV or other immunosuppressive conditions were not enrolled; trials are ongoing to assess safety and efficacy in these populations. Furthermore, the duration of efficacy of quadrivalent vaccine and the need for boosters is not known. An extension of the phase IIb study has shown the vaccine to be highly effective through 5 years[31] and in this same study, an antigen challenge of quadrivalent HPV6/11/16/18 vaccine was shown to stimulate an anamnestic response, the hallmark of a vaccine that offers long-lasting protection.[32] The planned 15-year follow-up of vaccinated individuals in northern Europe[33] and the planned long-term follow-up of 1500 9–15-year-old boys and girls enrolled in adolescent immunogenicity studies[29] should provide essential information on the durability of protection.

An increasing incidence of high-grade vulval intraepithelial neoplasia and vulval cancer has been noted over the past 30 years.[9,10,34] This trend is worrying because these cancers are not amenable to a screening programme. Whereas previously vulval cancer was seen almost exclusively in older women, recent studies have shown that 20% of these cancers now occur in women under 50 years.[9,10,35] In older women, vulval cancer occurs most commonly in association with non-HPV-related lichen sclerosus. Almost all vulval cancer cases among younger women are HPV related, with a high proportion attributed to HPV16.[10,36] In our study, 64% (21/33) of all cases of VIN2–3 seen in the placebo cohort were attributable to HPV16. A recent study by Srodon and colleagues[37] found HPV16 DNA in 91% (31/34) of cases of vulval carcinoma in situ. Other HPV types can be involved in vulval and vaginal neoplasia, although less frequently than HPV16.[38] We found HPV6 in five cases of VIN2–3 or VaIN2–3 that were not associated with HPV16 or HPV18. In women of this age-group, vulval carcinoma in situ can spontaneously regress.[39] However, there is a substantial risk of progression of HPV-related vulval carcinoma in situ to invasive cancer in women aged over 30 years.[16,39] The mean duration of follow-up in this study was

36 months. Even within this fairly short time, among young women who were naive to HPV16 or HPV18 at study entry, 29 placebo recipients developed an endpoint related to HPV16 or HPV18. In the intention-to-treat population, 31 women in the placebo arm developed an HPV16-related or HPV18-related high-grade lesion, corresponding to an incidence of up to 120 cases per 100 000 person-years.

Treatment for vulval and vaginal intraepithelial neoplasia can cause anxiety, depression, sexual dysfunction, and poor self-image. The treatment of choice for vulval intraepithelial neoplasia is surgery. Since the disease can be multifocal, surgery can be mutilating for these patients and adequate margins are sometimes impossible to achieve. Topical treatments—eg, imiquimod—are not highly effective and data are limited.[40,41] The recurrence rate of vulval intraepithelial neoplasia is high.[14,16,42] Women with vulval intraepithelial neoplasia have a substantial risk of developing invasive vulval cancer, even after treatment. In a prospective long-term follow-up study of 405 cases of vulval carcinoma in situ at a single centre, 15 (4%) of the patients developed squamous cell carcinoma of the vulva after treatment.[16] A recent meta-analysis of 3322 patients with vulval carcinoma in situ showed a 6·5% risk of squamous cell carcinoma of the vulva after treatment.[43] This risk is much higher than the risk for developing cancer after a cone biopsy for cervical intraepithelial neoplasia. Reich and coworkers[44,45] reported two series of treated patients with cervical intraepithelial neoplasia grade 3 with free or positive margins of the surgical specimen, respectively. Taking the two cohorts together, only six of 4807 women (0·12%) developed invasive cervical cancer during a mean follow-up of 18 years.[16] Kalliala and coworkers[46] reported 22 cases of invasive cervical cancer after treatment of cervical intraepithelial neoplasia in 7564 women (0·3%).[46] However, all women treated for anogenital precancers are at risk for developing vaginal cancer.[2] Our data show that vaccinating HPV-naive individuals is efficacious. Vaccination before sexual debut is preferable, since sexual transmission remains the main route of infection.

Precursors of vulval and vaginal cancers are often not recognised. Prevention of these conditions by a vaccine has the potential to lower a women's risk of developing vulval and vaginal cancer. Furthermore, mutilating surgery and repeated treatments can be avoided in the future. However, the real benefits to individuals and society will need to be weighed against the costs in a formal economic assessment of the vaccine.

In summary, these combined studies provide substantial evidence that a quadrivalent HPV L1 VLP vaccine is highly effective in preventing high-grade vulval and vaginal lesions associated with HPV16 or HPV18. The maximum effect of vaccination is expected in girls who are vaccinated in early adolescence, before exposure. The effect of vaccination in the general population of sexually experienced young women is expected to be lower initially, due to prevalent HPV infection. In the general population, the magnitude of reduction in overall rates of high-grade vulval and vaginal lesions associated with HPV16 or HPV18 is expected to increase over time, as the vaccine prevents new infections. This intervention could greatly reduce the morbidity, mortality and health-care costs associated with these diseases.

Contributors

EB, JB, CS, and MN managed the sponsor's operations. EAJ, SL, MH-A, CMW, GP, LAK, SMG, DMH, GWKT, DGF, and MS set up study sites and enrolled participants. DGF, DMH, EAJ, RWJ, GP, MS, and GWKT did colposcopic examinations. JB and FJT developed the PCR-based HPV6/11/16/18 detection assays and tested study cervicovaginal samples using the assays. MTE developed the anti-HPV6/11/16/18 immunoassays and tested study sera. OMB developed and implemented the data analysis plan. EAJ, JP, OMB and HLS drafted the manuscript, to which all authors contributed. The final version of the manuscript was seen and approved by all authors.

Conflict of interest statement

JP has received research grants from from Merck and Co through the University Central Hospital, Helsinki, to conduct clinical trial of this vaccine. He has received consulting fees or paid advisory boards from Merck & Co, and has received lecture fees from Merck and Co. LAK received grant support from her institution to do clinical trials for this vaccine. SMG has received advisory board fees and grant support from Commonwealth Serum Laboratories and GlaxoSmithKline, and lecture fees from Merck and Co. EAJ has a research contract with Merck Sharp and Dohme and GlaxoSmithKline and has received funding through the Medical University of Vienna, together with lecture fees and travel expenses from Sanofi Pasteur. SL has a research contract with Merck Sharp and Dohme, and has received funding through the Medical University of Vienna. MS has received consulting fees, advisory board fees, or lecture fees from Digene, Merck Frosst, GlaxoSmithKline, and Roche Diagnostics, and research grants from Merck Frosst and GlaxoSmithKline. MH-A has received fees and grant support from Merck and Co. DGF has acted as a consultant for Merck and Co and GlaxoSmithKline, and has acted as a speaker for Merck and Co. CMW has received research contracts for HPV vaccine studies from GlaxoSmithKline and Merck and Co. DMH has received study support for clinical trials, advisory board fees, and speaking fees from Merck and Co. GP has received research support, consulting fees, advisory board and lecture fees from Merck and Co. GWKT has received grant support through her institution to do clinical trials for this vaccine. JWB, FJT, OMB, MTE, HLS, JB, CS, EB, and MN are employees of Merck and Co and hold stock/stock options. RWJ declares no conflict of interest.

Acknowledgments

We thank all investigators and participants. Merck Research Laboratories, a division of Merck and Company, funded this study in its entirety. Part of these data were presented at the 42nd Annual Meeting of ASCO, June 2–6, 2006 (abstract 5011) and the 18th International Congress on Anti Cancer Treatment Meeting, February 6–9, 2007 (abstract EL 54).

References

1 Muñoz N, Bosch FX, de Sanjosé S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003; **348:** 518–27.

2 Daling JR, Madeleine MM, Schwartz SM, et al. A population-based study of squamous cell vaginal cancer: HPV and cofactors. *Gynecol Oncol* 2002; **84:** 263–70.

3 Madeleine MM, Daling JR, Carter JJ, et al. Cofactors with human papillomavirus in a population-based study of vulvar cancer. *J Natl Cancer Inst* 1997; **89:** 1516–23.

4 Partridge JM, Koutsky LA. Genital human papillomavirus infection in men. *Lancet Infect Dis* 2006; **6:** 21–31.

5 Goffin F, Mayrand MH, Gauthier P, et al. High-risk human papillomavirus infection of the genital tract of women with a previous history or current high-grade vulvar intraepithelial neoplasia. *J Med Virol* 2006; **78:** 814–19.

6 American Cancer Society. Detailed guide: vaginal cancer what are the risk factors for vaginal cancer? http://www.cancer.org/docroot/cri/content/cri_2_4_2x_what_are_the_risk_factors_for_vaginal_cancer_55.asp (accessed April 28, 2007).

7 Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB. Cancer incidence in 5 continents. IRAC Scientific Publication 155. Lyon, France: International Agency for Research on Cancer, 2002; 8.

8 Judson PL, Habermann EB, Baxter NN, Durham SB, Virnig BA. Trends in the incidence of invasive and in situ vulvar carcinoma. *Obstet Gynecol* 2006; **107:** 1018–22.

9 Joura EA, Losch A, Haider-Angeler MG, Breitenecker G, Leodolter S. Trends in vulvar neoplasia. Increasing incidence of vulvar intraepithelial neoplasia and squamous cell carcinoma of the vulva in young women. *J Reprod Med* 2000; **45:** 613–15.

10 Jones RW, Baranyai J, Stables S. Trends in squamous cell carcinoma of the vulva: the influence of vulvar intraepithelial neoplasia. *Obstet Gynecol* 1997; **90:** 448–52.

11 Hillemanns P, Wang X. Integration of HPV-16 and HPV-18 DNA in vulvar intraepithelial neoplasia. *Gynecol Oncol* 2006; **100:** 276–82.

12 Jones RW. Vulval intraepithelial neoplasia: current perspectives. *Eur J Gynaecol Oncol* 2001; **22:** 393–402.

13 Dodge JA, Eltabbakh GH, Mount SL, Walker RP, Morgan A. Clinical features and risk of recurrence among patients with vaginal intraepithelial neoplasia. *Gynecol Oncol* 2001; **83:** 363–69.

14 Herod JJ, Shafi MI, Rollason TP, Jordan JA, Luesley DM. Vulvar intraepithelial neoplasia: long term follow up of treated and untreated women. *Br J Obstet Gynaecol* 1996; **103:** 446–52.

15 Aho M, Vesterinen E, Meyer B, Purola E, Paavonen J. Natural history of vaginal intraepithelial neoplasia. *Cancer* 1991; **68:** 195–97.

16 Jones RW, Rowan DM, Stewart AW. Vulvar intraepithelial neoplasia: aspects of the natural history and outcome in 405 women. *Obstet Gynecol* 2005; **106:** 1319–26.

17 The FUTURE II Study Group. Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3, and adenocarcinoma in situ: a combined analysis of four randomised clinical trials. *Lancet* 2007 (in press).

18 Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N Engl J Med* 2007; **356:** 1928–43.

19 von Krogh G. Management of anogenital warts (condylomata acuminata). *Eur J Dermatol* 2001; **11:** 598–604.

20 Wiley DJ, Douglas J, Beutner K, et al. External genital warts: diagnosis, treatment and prevention. *Clin Infect Dis* 2002; **35** (suppl 2): S210–24.

21 Villa LL, Costa RLR, Petta CA, et al. 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. *Lancet Oncol* 2005; **6:** 271–78.

22 The FUTURE II Study Group. Quadrivalent HPV vaccine against human papillomavirus to prevent high-grade cervical lesions. *N Engl J Med* 2007; **356:** 1915–27.

23 Mao C, Koutsky LA, Ault KA, et al. Efficacy of human papillomavirus-16 vaccine to prevent cervical intraepithelial neoplasia: a randomized controlled trial. *Obstet Gynecol* 2006; **107:** 18–27.

24 Bryan J, Taddeo F, Skulsky D, et al. Detection of specific human papillomavirus types in paraffin-embedded sections of cervical carcinomas. *J Med Virol* 2006; **78:** 117–24.

25 Dias D, Van Doren J, Schlottmann S, et al. Optimization and validation of a multiplexed luminex assay to quantify antibodies to neutralizing epitopes on human papillomavirus 6, 11, 16 and 18. *Clin Diagn Lab Immunol* 2005; **12:** 959–69.

26 Chan IS, Bohidar NR. Exact power and sample size for vaccine efficacy studies. *Theory Meth* 1998; **27:** 1305–22.

27 Kalbfleisch JD, Prentice RL. The statistical analysis of failure time data. New York: John Wiley & Sons; 1980.

28 Block SL, Nolan T, Sattler C, et al. Comparison of the immunogenicity and reactogenicity of a prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in male and female adolescents and young adult women. *Pediatrics* 2006; **118:** 2135-45.

29 Reisinger KS, Block SL, Lazcano-Ponce E, et al. Safety and persistent immunogenicity of a quadrivalent human papillomavirus types 6, 11, 16, 18 L1 virus-like particle vaccine in preadolescents and adolescents: a randomized controlled trial. *Ped Infect Dis J* 2007; **26:** 201–09.

30 The American College of Obstetricians and Gynecologists. HPV vaccine-ACOG recommendations. http://www.acog.org/departments/dept_notice.cfm?recno=7&bulletin=3945 (accessed April 28, 2007).

31 Villa LL, Costa RLR, Petta CA, et al. High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. *Br J Cancer* 2006; **95:** 1459–66.

32 Olsson S-E, Villa LL, Costa R, et al. Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like-particle vaccine. *Vaccine* published online April 20, 2007. DOI:10.1016/j.vaccine.2007.03.049.

33 Lehtinen M, Herrero R, Mayaud P, et al. Chapter 28: Studies to assess the long-term efficacy and effectiveness of HPV vaccination in developed and developing countries. *Vaccine* 2006; **24** (suppl 3): S233–41.

34 Sturgeon SR, Brinton LA, Devesa SS, Kurman RJ. In situ and invasive vulvar cancer incidence trends (1973 to 1987). *Am J Obstet Gynecol* 1992; **166:** 1482–85.

35 Joura EA. Epidemiology, diagnosis and treatment of vulvar intraepithelial neoplasia. *Curr Opin Obstet Gynecol* 2002; **14:** 39–43.

36 Hampl M, Sarajuuri H, Wentzensen N, Bender HG, Kueppers V. Effect of human papillomavirus vaccines on vulvar, vaginal, and anal intraepithelial lesions and vulvar cancer. *Obstet Gynecol* 2006; **108:** 1361–68.

37 Srodon M, Stoler MH, Baber GB, Kurman RJ. The distribution of low and high-risk HPV types in vulvar and vaginal intraepithelial neoplasia (VIN and VaIN). *Am J Surg Pathol* 2006; **30:** 1513–18.

38 Toki T, Kurman RJ, Park JS, Kessis T, Daniel RW, Shah KV. Probable nonpapillomavirus etiology of squamous cell carcinoma of the vulva in older women: a clinicopathologic study using in situ hybridization and polymerase chain reaction. *Int J Gynecol Pathol* 1991; **10:** 107–25.

39 Jones RW, Rowan DM. Spontaneous regression of vulvar intraepithelial neoplasia 2–3. *Obstet Gynecol* 2000; **96:** 470–42.

40 Le T, Hicks W, Menard C, Hopkins L, Fung MF. Preliminary results of 5% imiquimod cream in the primary treatment of vulva intraepithelial neoplasia grade 2–3. *Am J Obstet Gynecol* 2006; **194:** 377–80.

41 Garland SM. Imiquimod. *Curr Opin Infect Dis* 2003; **16:** 85–89.

42 Hillemanns P, Wang X, Staehle S, Michels W, Dannecker C. Evaluation of different treatment modalities for vulvar intraepithelial neoplasia (VIN): CO(2) laser vaporization, photodynamic therapy, excision and vulvectomy. *Gynecol Oncol* 2006; **100:** 271–75.

43 van Seters M, van Beurden M, de Craen AJ. Is the assumed natural history of vulvar intraepithelial neoplasia III based on enough evidence? A systematic review of 3322 published patients. *Gynecol Oncol* 2005; **97:** 645–51.

44 Reich O, Pickel H, Lahousen M, Tamussino K, Winter R. Cervical intraepithelial neoplasia III: long-term outcome after cold-knife conization with clear margins. *Obstet Gynecol* 2001; **97:** 428–30.

45 Reich O, Lahousen M, Pickel H, Tamussino K, Winter R. Cervical intraepithelial neoplasia III: long-term follow-up after cold-knife conization with involved margins. *Obstet Gynecol* 2002; **99:** 193–96.

46 Kalliala I, Anttila A, Pukkala E, Nieminen P. Risk of cervical and other cancers after treatment of cervical intraepithelial neoplasia: retrospective cohort study. *BMJ* 2005; **331:** 1183–85.