Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24–45 years: a randomised, double-blind trial

Nubia Muñoz, Ricardo Manalastas Jr, Punee Pitisuttithum, Damrong Tresukosol, Joseph Monsonego, Kevin Ault, Christine Clavel, Joaquin Luna, Evan Myers, Sara Hood, Oliver Bautista, Janine Bryan, Frank J Taddeo, Mark T Esser, Scott Vuocolo, Richard M Haupt, Eliav Barr, Alfred Saah

Summary

Background **Although the peak incidence of human papillomavirus (HPV) infection occurs in most populations within 5–10 years of first sexual experience, all women remain at risk for acquisition of HPV infections. We tested the safety, immunogenicity, and efficacy of the quadrivalent HPV (types 6, 11, 16, 18) L1 virus-like-particle vaccine in women aged 24–45 years.**

Methods **Women aged 24–45 years with no history of genital warts or cervical disease were enrolled from community health centres, academic health centres, and primary health-care providers into an ongoing multicentre, parallel, randomised, placebo-controlled, double-blind study. Participants were allocated by computer-generated schedule to receive quadrivalent HPV vaccine (n=1911) or placebo (n=1908) at day 1, and months 2 and 6. All study site investigators and personnel, study participants, monitors, and central laboratory personnel were blinded to treatment allocation. Coprimary efficacy endpoints were 6 months' or more duration of infection and cervical and external genital disease due to HPV 6, 11, 16, 18; and due to HPV 16 and 18 alone. Primary efficacy analyses were done in a per-protocol population, but intention-to-treat analyses were also undertaken. This study is registered with ClinicalTrials.gov, number NCT00090220.**

Findings **1910 women received at least one dose of vaccine and 1907 at least one dose of placebo. In the per-protocol population, efficacy against the first coprimary endpoint (disease or infection related to HPV 6, 11, 16, and 18) was 90·5% (95% CI 73·7–97·5, four of 1615 cases in the vaccine group *vs* 41/1607 in the placebo group) and 83·1% (50·6–95·8, four of 1601 cases *vs* 23/1579 cases) against the second coprimary endpoint (disease or infection related to HPV 16 and 18 alone). In the intention-to-treat population, efficacy against the first coprimary endpoint was 30·9% (95% CI 11·1–46·5, 108/1886 cases *vs* 154/1883 cases) and against the second coprimary endpoint was 22·6% (–2·9 to 41·9, 90/1886 cases *vs* 115/1883 cases), since infection and disease were present at baseline. We recorded no vaccine-related serious adverse events.**

Interpretation **The quadrivalent HPV vaccine is efficacious in women aged 24–45 years not infected with the relevant HPV types at enrolment.**

Funding **Merck (USA).**

Lancet 2009; 373: 1949–57

Published Online June 2, 2009 DOI:10.1016/S0140-6736(09)60691-7

See Comment page 1921

National Institute of Cancer, Bogotá, Colombia (Prof N Muñoz MD, J Luna MD); **Infectious Disease Section, Department of Obstetrics and Gynecology, Philippine General Hospital, Manila, Philippines** (R Manalastas Jr MD); **Vaccine Trial Centre, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand** (P Pitisuttithum MD); **Department of Obstetrics and Gynecology, Chulalongkorn Hospital, Bangkok, Thailand** (D Tresukosol MD); **Federation Mutualiste Parisienne, Paris, France** (J Monsonego MD); **Department of Gynecology and Obstetrics, Emory University School of Medicine, Atlanta, GA, USA** (K Ault MD); **CHU Reims, Laboratoire Pol Bouin, Reims, France** (C Clavel PhD); **Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, USA** (E Myers MD); **and Merck, North Wales, PA, USA** (S Hood BS, O Bautista PhD, J Bryan PhD, F J Taddeo PhD, M T Esser PhD, S Vuocolo PhD, R M Haupt MD, E Barr MD, A Saah MD)

Correspondence to: Nubia Muñoz, 24 Quai Fulchiron, 69005 Lyon, France **nubia.munoz@free.fr**

Introduction

Studies of the natural history of human papillomavirus (HPV) infection and disease have shown that the peak incidence of HPV infection occurs in most populations within 5–10 years of first sexual experience (age 15–25 years). Women older than 25 years clearly remain at substantial risk for acquisition of HPV infections, although the extent to which infections occurring in mid-adult life are associated with subsequent risk of precancer and cancer is unclear.[1–3] In a cohort of 1600 women from Bogota, Colombia, the 5-year cumulative risk of cervical HPV infection of any type decreased from 42·5% in women aged 15–19 years to 22·0% in those aged 30–44 years,[4] showing a reduced, but not insignificant risk in the older cohort. A second peak in HPV DNA prevalence has been recorded in women in the fourth and fifth decades of life.[5] Whether this second peak is due to reactivation of latent infections, a cohort effect, or new HPV infections is not clear; however, the cohort study from Colombia lends support to the possibility of new HPV infections. The curve of incident HPV infections in these women showed a first peak in those younger than 25 years and a second peak after menopause.[4]

Changes in sexual behaviour during the past 30 years, characterised by rising age at first marriage and an increase in divorce rates, have led to more widespread premarital sexual intercourse and acquisition of new sexual partners around middle age, respectively.[6] Published work suggests that in the USA, nearly 40% of men and women have married and divorced by 55 years of age, and that more than 25% of these people have remarried at least once.[7] As the potential for HPV infection and disease exists in women in their third, fourth, and fifth decades of life, these women could benefit from prophylactic HPV vaccination.

Recent phase III trials (FUTURE I and II) undertaken in about 15 000 women aged 16–26 years from North America, Latin America, Asia, and Europe have shown that a prophylactic quadrivalent HPV (types 6, 11, 16, and 18) L1 virus-like-particle (VLP) vaccine adjuvanted with amorphous aluminium hydroxyphosphate sulphate to be highly effective in prevention of cervical, vulvar, or vaginal intraepithelial neoplasia related to HPV 6, 11, 16, or 18, as well as adenocarcinoma in situ in women who were naive to the respective HPV types at enrolment.[8,9] A high efficacy against genital warts related to HPV 6 and 11 was also seen.[8] Data from these trials indicated that women who were naive to all four vaccine HPV types (negative by both serological and DNA testing) before vaccination derive full benefit (ie, protection from disease caused by all four vaccine HPV types), whereas women who are infected with one or more vaccine HPV types before vaccination will derive partial benefit (ie, protection from the types that the participants were not infected with at time of vaccination).[10]

We undertook a phase III trial to assess the efficacy, safety, and immunogenicity of the prophylactic quadrivalent HPV vaccine in women aged 24–45 years.

Methods

Study design and participants

Between June, 18, 2004, and April 30, 2005, 3819 women between the ages of 24 years and 45 years were enrolled from 38 international study sites into an ongoing randomised, placebo-controlled, double-blind safety, immunogenicity, and efficacy study. Participants were enrolled from community health centres, academic health centres, and primary health-care providers in Colombia, France, Germany, Philippines, Spain, Thailand, and the USA. For all women enrolled, the duration of the study will be roughly 4 years. The present report represents a mean follow-up time of 2·2 years.

Women were eligible to participate in the study if they were not pregnant (as determined by a serum pregnancy test or urine pregnancy test sensitive to 25 IU β human chorionic gonadotropin done before every vaccination) and if they had not undergone hysterectomy. Participants enrolled were asked to use effective contraception until month 7 of the study. Women with a history of genital warts or present or past cervical disease were not eligible for enrolment. Women with any previous cervical surgical procedure and those having undergone a cervical biopsy within the past 5 years were also excluded. Additionally, women infected with HIV and those who were otherwise immunocompromised were not eligible for enrolment. Lifetime number of sexual partners was not an inclusion or exclusion criterion.

The institutional review board at every participating centre approved the protocol, and we obtained written informed consent from all participants. Studies were undertaken in accordance with applicable country or local requirements regarding ethics committee review, informed consent, and other statutes or regulations regarding the protection of the rights and welfare of human participants of biomedical research.

Procedures

Participants were stratified into two age groups (≤34 years and ≥35 years) and randomly assigned in an approximate 1:1 ratio to receive either amorphous aluminium hydroxyphosphate sulphate adjuvanted quadrivalent HPV (types 6, 11, 16, 18) L1 VLP vaccine (Gardasil/Silgard, Merck, Whitehouse Station, NJ, USA) or visually indistinguishable aluminium-containing placebo at day 1, and months 2 and 6. Details of the composition and production of the quadrivalent HPV vaccine have been published previously.[11]

A computer-generated allocation schedule was generated by the sponsor's Clinical Biostatistics department. After informed consent had been obtained and determination that all entry criteria were met, eligible women were randomly assigned to a vaccination group, stratified in roughly equal proportions between the two age groups within each study centre, by an interactive voice response system. All study site investigators and personnel, study participants, monitors, and central laboratory personnel were blinded to treatment allocation throughout the study; the treatment allocation was revealed to the study researchers after data collection and analyses were complete at end of study (last patient visit was completed in April, 2009).

The coprimary efficacy endpoints were the combined incidence of infection of at least 6 months' duration and cervical and external genital disease (including cervical, vulvar, or vaginal intraepithelial neoplasia; adenocarcinoma in situ; cervical, vulvar, or vaginal cancer; and genital warts) related to HPV 6, 11, 16, or 18; and to HPV 16 or 18 alone. The secondary efficacy endpoint was the combined incidence of infection related to HPV 6 or HPV 11 of 6 months' or more duration, and cervical and external genital disease. The first coprimary efficacy hypothesis was to be tested when 25 or more cases of the first coprimary efficacy endpoint and 14 or more cases of the second coprimary efficacy endpoint were observed (ie, fixed-event design). The second primary hypothesis was to be tested only if the first coprimary hypothesis test was successful. The secondary hypothesis was to be tested when 19 or more cases of the secondary efficacy endpoint were recorded, and only if both coprimary hypothesis tests were successful. Under an assumed incidence of 0·6, 0·2, and 0·6 per 100 person-years[12,13] corresponding to persistent infection and disease related to HPV 16, 18, and 6/11, respectively, the study with 3819 participants had 87% and 80% power to achieve success on the coprimary and secondary hypothesis, respectively, if the vaccine is at least 80% efficacious.

Infection of 6 months' or more duration was defined as detection of the same HPV type in cervicovaginal or anogenital swabs at two or more consecutive visits spaced

at least 6 months apart (1 month visit windows); or presence of cervical or genital disease associated with the relevant type with type-specific HPV DNA detected in cervicovaginal or anogenital swabs at the visit directly before or after the biopsy sample was taken. Disease was defined as a tissue sample diagnosed as cervical, vulvar, or vaginal intraepithelial neoplasia; adenocarcinoma in situ; genital warts; or cervical, vulvar, or vaginal cancer with type-specific HPV DNA detected as described previously.[8]

For the ascertainment of disease, a complete gynaecological examination was done at day 1 and months 7, 12, 24, 36, and 48 that included a pelvic examination (both speculum and bimanual examinations). External genital inspections were done with a magnifying glass at day 1, and at months 7, 12, 18, 24, 30, 36, 42, and 48. Labial/vulvar/perineal and perianal swabs and endocervical and ectocervical swabs for HPV multiplex PCR testing were obtained at day 1 and months 7, 12, 18, 24, 30, 36, 42, and 48. ThinPrep (Cytyc, Boxborough, MA, USA) Pap testing for cytology took place during these visits. Once received, cytology specimens were assessed with the Bethesda System–2001.[14] Colposcopy referral was algorithm-based. Biopsy material was first read for clinical management by pathologists at a central laboratory (Diagnostic Cytology Laboratories, Indianapolis, IN, USA), and then read for endpoint determination by a blinded panel of four pathologists. When indicated, definitive therapy was undertaken according to local standards of care.

The primary immunogenicity objectives were to assess the kinetics and age dependence of anti-HPV 6, 11, 16, and 18 responses after administration of a three-dose regimen of quadrivalent HPV vaccine; and to compare anti-HPV 6, 11, 16, and 18 responses after administration of a three-dose regimen of quadrivalent HPV vaccine in HPV-naive women aged 24–45 years enrolled in this protocol and HPV-naive women aged 16–23 years from protocols 013[8] and 015.[9] Serum for immunogenicity testing was collected before vaccination on day 1, and at months 7, 12, 24, 36, and 48. We analysed antibody responses and the proportion of participants seroconverting for vaccine type epitope-specific neutralising anti-HPV antibodies with a competitive Luminex-based immunoassay (developed by Merck Research Laboratories using technology from the Luminex corporation as described previously).[15]

The primary safety objective was to show that a three-dose regimen of quadrivalent HPV vaccine was generally well tolerated in women aged 24–45 years. We gathered information about adverse events from participants by general questioning at study visits and by use of a vaccine report card. This card was provided to the participant at every vaccination visit to record temperatures and local and systemic adverse events.

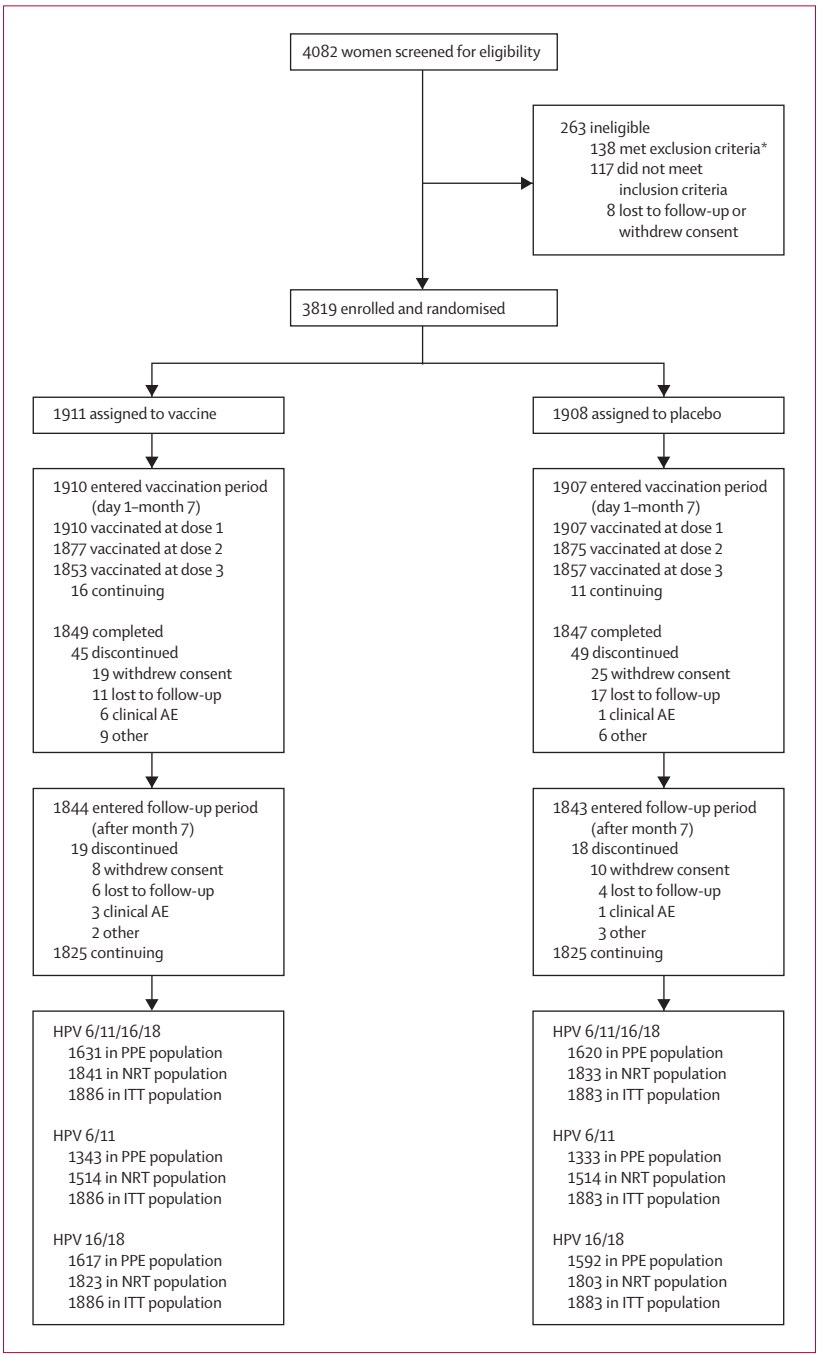


***Figure 1*: Trial profile**
Completed refers to women who completed the three-dose vaccine regimen or placebo regimen during the vaccination period. AE=adverse event. HPV=human papillomavirus. PPE=per-protocol efficacy. NRT=naive to the relevant HPV type. ITT=intention-to-treat. *Includes 31 women who were excluded because they had a history of disease, and 44 excluded because they had a condition that the investigator felt might interfere with the evaluation of the study objectives.

Statistical analysis

Primary tests of efficacy hypotheses were based on HPV type-specific per-protocol efficacy (PPE) analyses. To be eligible for this population, women must have been seronegative to the relevant HPV type at day 1 and PCR negative to that type in cervicovaginal swabs or biopsy samples, or both, from day 1 until month 7. Additionally, to be included in the PPE assessment, participants must

	Vaccine (n=1911)	Placebo (n=1908)	Total (N=3819)
Age (years)			
Mean (SD)	34·3 (6·3)	34·3 (6·3)	34·3 (6·3)
Median (range)	35 (24–45)	34 (21–46)	34 (21–46)
Race/ethnic origin			
Asian	596 (31·2%)	596 (31·2%)	1192 (31·2%)
Black	100 (5·2%)	82 (4·3%)	182 (4·8%)
Hispanic	822 (43·0%)	827 (43·3%)	1649 (43·2%)
Native American	2 (0·1%)	1 (0·1%)	3 (0·1%)
White	388 (20·3%)	397 (20·8%)	785 (20·6%)
Other	3 (0·2%)	5 (0·3%)	8 (0·2%)
Region			
Asia-Pacific	591 (30·9%)	591 (31·0%)	1182 (31·0%)
Europe	245 (12·8%)	237 (12·4%)	482 (12·6%)
Latin America	804 (42·1%)	806 (42·2%)	1620 (42·2%)
North America	271 (14·2%)	274 (14·4%)	545 (14·3%)
Smoking status			
Current smoker	339 (17·7%)	332 (17·4%)	671 (17·6%)
Ex-smoker	159 (8·3%)	148 (7·8%)	307 (8·0%)
Never smoked	923 (48·3%)	935 (49·0%)	1858 (48·6%)
Missing or unknown	490 (25·6%)	493 (25·8%)	983 (25·7%)
Contraceptive use			
Barrier	441 (23·1%)	425 (22·3%)	866 (22·6%)
Behaviour	165 (8·6%)	184 (9·6%)	349 (9·1%)
Hormonal	596 (31·2%)	591 (31·0%)	1187 (31·1%)
Other*	748 (39·2%)	749 (39·3%)	1497 (39·2%)
Baseline HPV positivity† to HPV 6, 11, 16, or 18			
By serology	575 (30·1%)	560 (29·4%)	1135 (29·8%)
By PCR	159 (8·4%)	139 (7·4%)	298 (7·9%)
By serology or PCR	635 (33·5%)	617 (32·8%)	1252 (33·2%)

Data are number (%) unless otherwise indicated. HPV=human papillomavirus.
*Other includes female (461 vaccine, 474 placebo) or male (69 vaccine, 65 placebo) sterilisation and intrauterine devices (220 vaccine, 210 placebo).
†In women with non-missing data, positive by serology was defined as having an anti-HPV competitive Luminex-based immunoassay titre greater than or equal to the serostatus cutoff values of 20 milli Merck Units (mMU; arbitrary value for measuring HPV antibody responses in sera) per mL, 16 mMU/mL, 20 mMU/mL, or 24 mMU/mL for HPV 6, 11, 16, or 18, respectively; negative by serology was defined as having titres less than these cutoffs. Positive by PCR was defined as having a positive PCR result at day 1 on at least one of the following: labial/vulvar/perineal/perianal swabs, endocervical or ectocervical swabs, or (if obtained) external genital biopsy specimens, or cervical biopsy specimens; negative by PCR was defined as having a negative PCR result on day 1 on all the above factors.

Table 1: **Baseline characteristics of enrolled participants**

have received all three vaccinations within 1 year, and have one or more follow-up visits after month 7. Protocol violators were not included. Cases were counted starting at month 7. The statistical criterion for study success required that the lower bound of the confidence interval for vaccine efficacy excluded 0% for each of the primary and secondary efficacy analysis. Analyses were also undertaken in two supportive populations: a population naive to the relevant type (NRT) and an intention-to-treat (ITT) population. The NRT population was a modified PPE population that included women who were naive to a vaccine HPV type at day 1, received at least one dose of vaccine or placebo, and had one or more follow-up visits after day 1. Cases were counted starting at day 1. The ITT population included all women who received at least one dose of vaccine or placebo and had one or more follow-up visits after day 1. Both protocol violators and those with pre-existing HPV infections were included in intention-to-treat analyses. Cases were counted starting at day 1.

This study is registered with ClinicalTrials.gov, number NCT00090220.

Role of the funding source

The studies were designed by the sponsor in collaboration with external investigators and an external data and safety monitoring board. The sponsor collated the data, monitored the conduct of the study, did the statistical analysis, and coordinated the writing of the report 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 access to data and took part in the decision to submit for publication.

Results

Figure 1 shows the trial profile. Of the 4082 participants who were screened for eligibility, 3819 were enrolled into the study and were randomly assigned to receive either quadrivalent HPV vaccine (n=1911) or placebo (n=1908). 263 women were screened and not randomly assigned to a group (figure 1). Two women were randomly assigned but not vaccinated. 85% of all vaccinated participants were included in the PPE analysis specific to one or more HPV vaccine type (n=1631). 94 women (3%) discontinued the study during the vaccination period, mostly because of loss to follow-up or withdrawal of consent. The proportion of participants not completing the vaccination regimen, and the distribution of reasons for discontinuation from the study, were generally similar in the vaccine and placebo groups (figure 1). Few subjects in either group discontinued because of an adverse event (five in the vaccine group *vs* one in the placebo group).

Baseline demographic characteristics were generally much the same between women in the vaccine and placebo groups (table 1). Although Asian and white ethnic origins were well represented, the largest proportion of study participants were Hispanic, owing to a large enrolment from Colombia. The most common contraceptive methods were barrier, hormonal, and other methods (including female and male sterilisation and intrauterine devices; table 1). About a third of women (33·2%) were positive to HPV 6, 11, 16, or 18 at baseline by serological (via competitive Luminex-based immunoassay) or DNA testing (via PCR); however, only 7·9% were infected with a vaccine HPV type at baseline as determined by DNA testing alone (table 1). 3455 (90%) women enrolled were susceptible to

three or four vaccine HPV types (2565 [67%] were naive via PCR and serology to all four vaccine HPV types; data not shown). Additionally, most of the women positive to vaccine-type HPV DNA were positive to only one HPV type (25 [1%] were infected with exactly two vaccine types, three [<1%] were infected with exactly three vaccine HPV types, and none were infected with all four types).

Almost all women had had sexual intercourse before enrolment, and the mean age of first sexual experience was 19 years (SD 3·7; table 2). A large proportion of enrolled women were married (in their first marriage) at baseline, followed by those who were living in a permanent relationship, and those who had never been married and were not living in a permanent relationship (table 2).

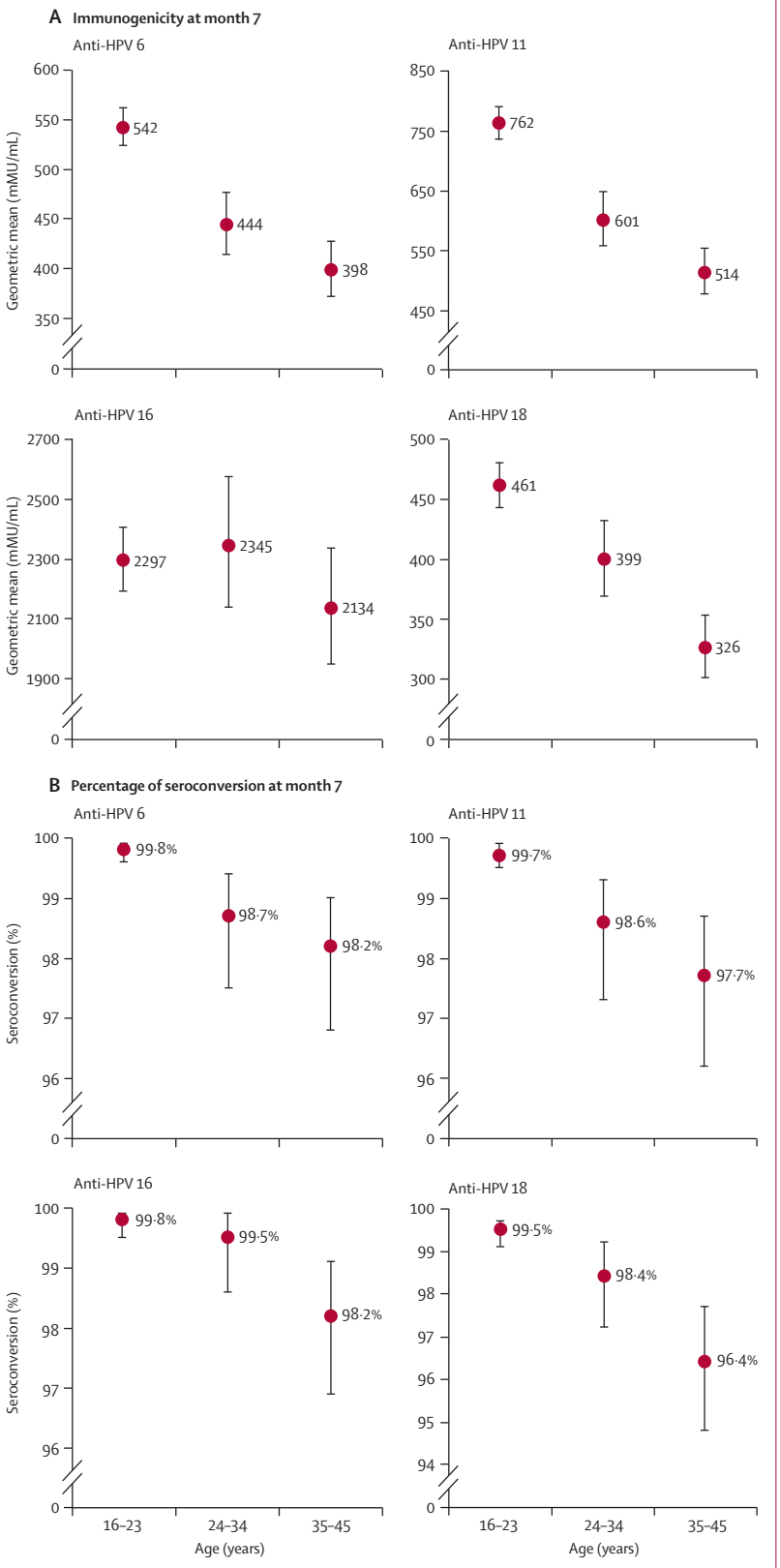
Vaccine-induced antibody titres against HPV 6, 11, 16, and 18 were similar in women aged 24–34 years and 35–45 years (figure 2A). Compared with immunological data from women aged 16–23 years who were enrolled in the quadrivalent HPV vaccine programme, we noted that the antibody responses in women aged 25–45 years were comparable for HPV 16, and slightly lower for HPV 6, 11, and 18 (figure 2A). Almost all women aged 24–45 years seroconverted (as defined by our competitive Luminex-based immunoassay) for vaccine HPV types by month 7 (figure 2B). Overall, in women aged 24–45 years, 1242 (98%) were anti-HPV 6 seropositive, 1238 (98%) were anti-HPV 11 seropositive, 1264 (99%) were anti-HPV 16 seropositive, and 1406 (97%) were anti-HPV 18 seropositive at month 7. We noted small differences in the percentage of participants who were seropositive for vaccine HPV types at month 7 when stratified by age, with an expected trend towards slightly reduced immune responses in the older cohort of women (data not shown). Generally, women who were seropositive to a particular vaccine HPV type at enrolment had higher antibody titres for that type at 24 months than did those who were naive to that type at enrolment, probably due to immune memory (data not shown).

In women aged 24–45 years in PPE populations specific to HPV vaccine type, vaccine efficacy against the first coprimary endpoint (combined incidence of infection of at least 6 months' duration and cervical and external genital disease related to HPV 6, 11, 16, and 18) was 90·5% (95% CI 73·7–97·5, consisting of four cases in the vaccine group and 41 cases in the placebo group; table 3). Results were similar between both protocol-defined age strata. Vaccine efficacy in the prevention of vaccine-type-related infection alone and disease alone (cervical intraepithelial neoplasia and external genital lesions) in the PPE population was 92·6% (95% CI 76·9–98·5) and 92·4% (49·6–99·8), respectively (infection: three vaccine and 40 placebo cases; disease: one vaccine and 13 placebo cases). Vaccine efficacy against the second coprimary endpoint (combined incidence of infection of ≥6 months' duration of cervical and external genital disease related to HPV 16 and 18) was 83·1% (50·6–95·8, consisting of four cases in the vaccine group and 23 cases in the placebo group; table 3). These results were also similar between both protocol-defined age groups (table 3). Efficacy against the secondary endpoint (combined incidence of

	Vaccine (n=1911)	Placebo (n=1908)	Total (N=3819)
Sexual history			
Number with available data	1910	1907	3817
Have never had sexual intercourse	1 (0·1%)	2 (0·1%)	3 (0·1%)
Have had sexual intercourse	1909 (99·9%)	1905 (99·9%)	3814 (99·9%)
Age of first sexual experience (years)			
Mean (SD)	19 (3·7)	19 (3·7)	19 (3·7)
Median (IQR)	18 (17–21)	18 (16–21)	18 (17–21)
Marital status*			
Never married	344 (18·0%)	335 (17·6%)	679 (17·8%)
Separated or divorced	155 (8·1%)	127 (6·7%)	282 (7·4%)
Widowed	12 (0·6%)	19 (1·0%)	31 (0·8%)
Living in a permanent relationship	530 (27·7%)	522 (27·4%)	1052 (27·5%)
Married, in first marriage	769 (40·2%)	791 (41·5%)	1560 (40·8%)
Divorced and remarried	94 (4·9%)	107 (5·6%)	201 (5·3%)
Widowed and remarried	7 (0·4%)	7 (0·4%)	14 (0·4%)
LSP at enrolment			
Unknown†	1 (0·1%)	0 (0·0%)	1 (0·0%)
0	0 (0·0%)	2 (0·1%)	2 (0·0%)
1	719 (37·6%)	751 (39·4%)	1470 (38·5%)
2	385 (20·2%)	362 (19·0%)	747 (19·6%)
3	229 (12·0%)	223 (11·7%)	452 (11·8%)
4	142 (7·4%)	130 (6·8%)	272 (7·1%)
>4	433 (22·7%)	437 (22·9%)	870 (22·8%)
Median (IQR)	2 (1–4)	2 (1–4)	2 (1–4)
New male or female sexual partners in 6 months before study			
Unknown†	4 (0·2%)	3 (0·2%)	7 (0·2%)
0	1737 (90·9%)	1728 (90·6%)	3465 (90·7%)
1	143 (7·5%)	151 (7·9%)	294 (7·7%)
2	15 (0·8%)	16 (0·8%)	31 (0·8%)
3	6 (0·3%)	6 (0·3%)	12 (0·3%)
4	3 (0·2%)	1 (0·1%)	4 (0·1%)
>4	1 (0·1%)	0 (0·0%)	1 (0·0%)
Pregnancy history*			
Unknown	1 (0·1%)	1 (0·1%)	2 (0·1%)
0	348 (18·2%)	367 (19·2%)	715 (18·7%)
1	352 (18·4%)	326 (17·1%)	678 (17·8%)
2	484 (25·3%)	503 (26·4%)	987 (25·8%)
3	378 (19·8%)	361 (18·9%)	739 (19·4%)
4	190 (9·9%)	195 (10·2%)	385 (10·1%)
5	78 (4·1%)	88 (4·6%)	166 (4·3%)
≥6	80 (4·2%)	67 (3·5%)	147 (3·8%)

Data are number (%) unless otherwise indicated. LSP=lifetime number of male or female sexual partners. *Percentages calculated on the basis of the number of allocated participants in each vaccination group. Percentages for all other categories calculated as 100×(number in category/number with sexual history data at enrolment). †Unknown means that the woman has had at least one sexual partner before study entry but did not remember or did not document their lifetime number of sexual partners.

Table 2: **Summary of sexual history at enrolment**



infection of ≥6 months' duration and cervical and external genital disease related to HPV 6 and 11) was 100·0% (95% CI 79·0–100·0, no cases in the vaccine group and 19 cases in the placebo group).

For women aged 24–45 years in the NRT and ITT populations (supportive analyses), vaccine effectiveness against the combined incidence of infection of 6 months' or more duration and cervical and external genital disease related to HPV 6, 11, 16, and 18 was 74·6% (95% CI 58·1–85·3, 20 cases in the vaccine group and 77 in the placebo group) and 30.9% (11·1–46·5, 108 cases in the vaccine group and 154 in the placebo group), respectively (table 3). Vaccine effectiveness in the prevention of vaccine-type-related disease alone (cervical intraepithelial neoplasia and external genital lesions) in the NRT and ITT populations was 82·0% (47·0–95·5) and 29·7% (–11·4 to 56·1), respectively (NRT population: four vaccine and 22 placebo cases; ITT population: 34 vaccine and 48 placebo cases).

The proportion of women who reported a serious adverse event on day 1–15 after any vaccination was comparable between the vaccine (three of 1889) and placebo (seven of 1886) groups (table 4). Injection-site adverse events were mainly responsible for the slight increase in adverse events recorded in the vaccine group (table 4). In the vaccine group, serious adverse events included rhinitis, vertigo, and tension headache. In the placebo group, serious adverse events included gastroenteritis, pulmonary tuberculosis, gastrointestinal tuberculosis, anaemia, pyelonephritis (two cases), ectopic pregnancy, and hepatitis.

Discussion

The PPE analysis for the use of a prophylactic quadrivalent HPV vaccine in adult women aged 24–45 years shows that vaccine efficacy against infection of at least 6 months' duration and cervical and external genital disease is high (mostly due to efficacy against infection). Differences in efficacy in younger and older women are probably due to more HPV infections occurring in younger women. The quadrivalent HPV vaccine already has a proven benefit in women and girls aged 9–26 years.[8,9] The current study shows that susceptible women 24–45 years of age could also benefit from vaccination.

To confidently extrapolate efficacy results from the current trial to cervical cancer prevention in general,

***Figure 2*: Immunogenicity (A) and percentage of seroconversion (B) at month 7 for vaccine HPV types in participants aged 16–45 years**
Data are from a per-protocol immunogenicity population that includes all participants who were not general protocol violators, received all three vaccinations within acceptable day ranges, were seronegative at day 1 and PCR negative from day 1 to month 7 for the relevant HPV type or types, and had a month 7 serum sample collected within an acceptable day range. Participants aged 16–23 years are from the combined quadrivalent HPV vaccine phase III FUTURE programme.[8,9] Seropositivity is defined as a competitive Luminex-based immunoassay geometric mean titre for HPV 6, 11, 16, and 18 of at least 20 milli Merck Units (mMU)/mL, 16 mMU/mL, 20 mMU/mL, and 24 mMU/mL, respectively. (An mMU is an arbitrary value for measuring HPV antibody responses in sera.) HPV=human papillomavirus.

	Vaccine (n=1910)			Placebo (n=1907)			Efficacy (95% CI)	p value
	n	Cases	Rate	n	Cases	Rate		
Per-protocol population*								
HPV 6/11/16/18-related (all women)	1615	4	0·1	1607	41	1·5	90·5% (73·7 to 97·5)	<0·0001
Women aged 24–34 years	792	2	0·2	792	24	1·8	91·8% (67·1 to 99·1)	..
Women aged 35–45 years	823	2	0·1	815	17	1·3	88·6% (51·9 to 98·7)	..
HPV 16/18-related (all women)	1601	4	0·1	1579	23	0·9	83·1% (50·6 to 95·8)	0·0001
Women aged 24–34 years	784	2	0·2	774	13	1·0	85·0% (33·8 to 98·4)	..
Women aged 35–45 years	817	2	0·1	805	10	0·7	80·6% (9·1 to 97·9)	..
HPV 6/11-related (all women)	1329	0	0·0	1323	19	0·9	100% (79·0 to 100)	<0·0001
Women aged 24–34 years	636	0	0·0	653	12	1·1	100% (63·7 to 100)	..
Women aged 35–45 years	693	0	0·0	670	7	0·6	100% (33·6 to 100)	..
Naive to the relevant type population†								
HPV 6/11/16/18-related (all women)	1841	20	0·5	1833	77	2·0	74·6% (58·1 to 85·3)	..
Women aged 24–34 years	914	11	0·6	920	54	2·8	79·8% (61·0 to 90·5)	..
Women aged 35–45 years	927	9	0·4	913	23	1·2	62·3% (15·4 to 84·6)	..
HPV 16/18-related (all women)	1823	14	0·4	1803	48	1·2	71·6%(47·6 to 85·5)	..
Women aged 24–34 years	904	9	0·5	901	33	1·7	73·0% (42·3 to 88·6)	..
Women aged 35–45 years	919	5	0·2	902	15	0·8	68·0% (7·3 to 90·9)	..
HPV 6/11-related (all women)	1514	6	0·2	1514	30	0·9	80·2% (51·7 to 93·3)	..
Women aged 24–34 years	735	2	0·1	770	22	1·3	90·6% (61·7 to 98·9)	..
Women aged 35–45 years	779	4	0·2	744	8	0·5	52·8%(–76·1 to 89·6)	..
Intention-to-treat population‡								
HPV 6/11/16/18-related (all women)	1886	108	2·7	1883	154	3·9	30·9% (11·1 to 46·5)	..
Women aged 24–34 years	937	71	3·7	944	94	4·9	23·7% (–4·9 to 44·7)	..
Women aged 35–45 years	949	37	1·8	939	60	3·0	40·7% (9·2 to 61·7)	..
HPV 16/18-related (all women)	1886	90	2·2	1883	115	2·9	22·6% (–2·9 to 41·9)	..
Women aged 24–34 years	937	57	2·9	944	70	3·6	17·7% (–18·4 to 43·0)	..
Women aged 35–45 years	949	33	1·6	939	45	2·2	28·9% (–13·9 to 56·1)	..
HPV 6/11-related (all women)	1886	24	0·6	1883	45	1·1	47·1% (11·4 to 69·2)	..
Women aged 24–34 years	937	17	0·8	944	28	1·4	38·4% (–16·5 to 68·4)	..
Women aged 35–45 years	949	7	0·3	939	17	0·8	60·1% (–1·3 to 86·0)	..

HPV=human papillomavirus. Rate=incidence rate per 100 person-years at risk. *Participants were seronegative to the relevant vaccine HPV type at day 1 and PCR negative to that type in cervicovaginal swabs or biopsy samples, or both, from day 1 to month 7. Additionally, women must have received all three vaccinations within 1 year, and have one or more follow-up visits after month 7. Protocol violators were generally not included. Cases were counted starting at month 7. †Women were naive to the relevant vaccine HPV type at day 1, received at least one dose of vaccine or placebo, and had one or more follow-up visits after day 1. Cases were counted starting at day 1. ‡Women received at least one dose of vaccine or placebo and had one or more follow-up visits after day 1. Both protocol violators and those with pre-existing HPV infections were included in intention-to-treat analyses. Cases were counted starting at day 1. §Women are counted once in each applicable endpoint category, but could be included in more than one category.

Table 3: **Efficacy against the combined incidence of vaccine-type-related infection of at least 6 months' duration, cervical intraepithelial neoplasia, and external genital lesions§**

the endpoints used to assess efficacy should be robust. In the pivotal phase III FUTURE efficacy trials of the quadrivalent HPV vaccine (in 16–26-year-old women), high efficacy was shown with high-grade cervical, vulvar, and vaginal lesions and genital warts as the primary endpoints[8,9] (WHO's recommended clinical endpoint for establishment of the efficacy of prophylactic HPV vaccines).[16,17] Additionally, in an analysis of participants enrolled in the FUTURE I trial,[8] the positive predictive value of HPV infection for a diagnosis of cervical intraepithelial neoplasia 2/3 related to either HPV 16 or HPV 18 was no worse for infections of less than 12 months (>0 to <6 months, and >6 to <12 months) than for infections of more than 12 months (data not shown). In the present report, we used a combined endpoint of HPV infection of 6 months' or more duration and HPV-related anogenital disease. With consideration of the previously shown efficacy of the vaccine against disease related to HPV 6, 11, 16, and 18, the inclusion of infection into a composite endpoint allowed for a more rapid assessment of the efficacy of the vaccine in women aged 24–45 years, due to the sometimes lengthy interval between HPV infection and disease. For this reason, the current study was designed as an efficacy bridging study. Although the current study was powered to assess the composite endpoint of infection and disease, most of the endpoints seen were infection (without disease).

	Vaccine (n=1908)	Placebo (n=1902)
Participants with follow-up	1889	1886
One or more adverse events	1642 (86·9%)	1532 (81·2%)
Injection-site adverse events	1450 (76·8%)	1212 (64·3%)
Systemic adverse events	1118 (59·2%)	1131 (60·0%)
Vaccine-related* adverse events	1565 (82·8%)	1389 (73·6%)
Injection-site adverse events	1449 (76·7%)	1212 (64·3%)
Systemic adverse events	745 (39·4%)	695 (36·9%)
Serious adverse events†	3 (0·2%)	7 (0·4%)
Serious vaccine-related* adverse events	0	0
Discontinued due to an adverse event‡	5 (0·3%)	1 (0·1%)
Discontinued due to a vaccine-related* adverse event	5 (0·3%)	1 (0·1%)
Discontinued due to a serious adverse event	0	0
Discontinued due to a serious vaccine-related* adverse event	0	0

Data are number (%) unless otherwise indicated. *Determined by the investigator to be possibly, probably, or definitely related to vaccine. †In the vaccine group, serious adverse events included one case of rhinitis, vertigo, and tension headache. In the placebo group, serious adverse events included one case of gastroenteritis, peritoneal tuberculosis, gastrointestinal tuberculosis, anaemia, pyelonephritis (two cases), ectopic pregnancy, and hepatitis. ‡Vaccine-related adverse events leading to withdrawal in the vaccine group included one case each of hypersensitivity, pharyngeal oedema, urticaria, mouth ulceration, and injection site pain or swelling. The vaccine-related adverse event leading to withdrawal in the placebo group was dizziness or fatigue.

Table 4: **Summary of clinical adverse events (days 1–15 after any vaccination visit)**

Ethically, use of high-grade genital lesions as endpoints in future placebo-controlled trials with prophylactic HPV vaccines might no longer be appropriate. Endpoints based on HPV infection might be sufficient. Women in the placebo group of this trial are being offered protection through screening every 6 months, and the vaccine will be offered to all participants at the end of the trial. We also detected high efficacy in the PPE population when an analysis of vaccine-type-related disease alone (cervical intraepithelial neoplasia and external genital lesions) was done. However, most of the cervical HPV-related disease was diagnosed as CIN 1, which is considered a morphological manifestation of productive HPV infection. We noted reduced efficacy in the supportive analyses of the NRT and ITT populations, although not unexpectedly because of the duration of follow-up. Case counting for these two populations began at day 1, rather than on month 7 as for the PPE population. Additionally, the ITT population included women with pre-existing infection or disease and those who violated the protocol. The estimates of efficacy are expected to increase over time as the prevalent cases are exhausted in the vaccine group and continue to accrue in the placebo group.

Maximum effect from prophylactic HPV vaccination programmes will be achieved in women who are susceptible to infection and disease related to vaccine HPV types (those not previously exposed).[18] Notably, most adult women enrolled in the current study remained susceptible to vaccine HPV types at entry. Almost all women enrolled were susceptible to three or four vaccine HPV types, and about a third were positive to HPV 6, 11, 16, or 18 at baseline by serological or DNA testing; therefore about two-thirds were susceptible to all four vaccine HPV types. Most women who were HPV positive were positive to only one HPV type, meaning that the quadrivalent HPV vaccine could still potentially benefit these women via protection against vaccine HPV types with which they are not infected with.[10]

These data, together with the demonstration of benefit in susceptible women independently of the woman's age, raise the issue of who should be vaccinated. Slight differences in efficacy between younger and older women were probably due to more HPV infections (DNA) in the younger women. Furthermore, differences in efficacy against 6/11-related and 16/18-related endpoints can be explained by case counting. HPV 16 and 18 are fairly common and although many other HPV types cause cervical and other lesions, if HPV 16 or 18 was found in a lesion (whether causative or not) then that person would be regarded as a case. HPV 6 and 11 cause almost all external genital lesions, which, compared with the lower prevalence of these types, suggests that these types are less common in a lesion in which they are not causative, leading to greater relative efficacy compared with HPV 16 and 18 efficacy. Lower effectiveness (about 30%) detected in the mixed population (susceptible women and those who have already acquired HPV infection or HPV-associated disease) suggests that the public health effect of vaccinating women aged 25–45 years will be smaller than that recorded after vaccinating susceptible adolescents. This notion will be assessed in future cost–benefit analyses.

Our study has several limitations. First, the amount of disease that will be prevented by vaccinating women between the ages of 25 years and 45 years outside of a clinical trial is unknown. HPV infections that occur in women of this age could lead to high-grade lesions and cancer at different rates than might infections that occur shortly after first sexual experience. Second, because only 50–70% of HPV infections result in detectable anti-HPV responses, the baseline serology test could have underestimated previous exposure to HPV 6, 11, 16, or 18 in the current study population. Third, the study used a fixed-event design, and we detected the required number of endpoints roughly halfway through the 4-year study. Thus, our results are based on a mean follow-up time of only 2 years, which limited the number of endpoints based on incident genital disease. Lastly, specific exclusion criteria might indicate that women in our study were at somewhat lower risk of acquiring HPV than were those in the general population (disease history, etc). However, when we compare the baseline prevalence and incidence of HPV 16/18 infection in our placebo group with those reported in a cohort of women of similar age,[4] we noted no differences (data not shown).

Our results are generalisable to women aged 24–45 years in the general population who have had no

(recent) cervical disease and no previous history of external genital disease. Generalisability in this population is supported by the standard screening and management procedures that were used, and by the fact that the number of lifetime sexual partners was not an inclusion or exclusion criterion.

Contributors

EB, RH, SH, and AS managed the sponsor's operations. JM, RM, PP, and DT set up study sites and enrolled participants into the study. KA, EM, CC, JL, and NM helped draft the protocol. JB and FJT developed the PCR-based HPV 6/11/16/18 detection assays and tested the genital swab and biopsy samples with the assays. MTE developed the anti-HPV 6/11/16/18 immunoassays and tested study sera. OB developed and instituted the data analysis plan. NM and SV drafted the report, to which all others contributed and approved before submission.

Study investigators

Colombia Nathalia Amaya, Alfonso Correa, Ivette Maldonado, Natascha Ortiz, Manuel Antonio Plata; *France* Jean-Paul Bory, Elisabeth Rohmer-Meyer, Gilles Mirguet, Didier Riethmuller; *Germany* Elisabeth Barthell, Anna-Margarete Funke, Fritz Jaenicke, Werner Lichtenegger, Silke Schmidt-Petruschkat; *Philippines* Efren Domingo; *Spain* Xavier Castellsague; *Thailand* Saowanee Karnplumjid, Jittima Manonai, Suwanit Therasakvichya; *USA* David Baker, Karl Beutner, Stan Block, Ramon Cestero, Daron Ferris, Larry Glazerman, Sharmila Makhija, William Nebel, Larry Seidman, Malcolm Sperling, Suzanne Trupin, Molly Yardley, Peter Zedler, Wallace Wilkerson, Darron Brown.

Conflicts of interest

NM has received honoraria from Merck and Sanofi-Pasteur MSD, and is a member of the Merck global advisory board for HPV vaccine as well as a member of Sanofi-Pasteur MSD HPV steering committee. JL has received travel, speaker, and investigator grants from Sanofi-Pasteur MSD. JM has undertaken HPV vaccine studies for Merck and GlaxoSmithKline, and is on the medical advisory board for GlaxoSmithKline, Geneprobe, Sanofi-Pasteur MSD, Roche, and Abbott diagnostics. KA has undertaken HPV vaccine studies for Merck and GlaxoSmithKline, and has acted as a consultant to Merck. EM has served as a consultant to Merck. NM, JL, KA, JM, EM, and CC are members of the Merck HPV steering committee. OB, SH, JB, FJT, MTE, SV, EB, AS, and RH are employees of Merck and potentially own stock and/or stock options in the company.

Acknowledgments

Merck Research Laboratories, a Division of Merck, funded this study in its entirety. We thank all study participants. These data were presented, in part, at the 24th International Papillomavirus Conference and Clinical Workshop, Nov 3–9, 2007, Beijing, China (abstr PA1-04).

References

1 Woodman CBJ, Collins S, Winter H, et al. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. *Lancet* 2001; **357:** 1831–36.

2 Collins S, Mazloomzadeh S, Winter H, et al. High incidence of cervical human papillomavirus infection in women during their first sexual relationship. *Br J Obstet Gynaecol* 2002; **109:** 96–98.

3 Winer RL, Lee S-K, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. *Am J Epidemiol* 2003; **157:** 218–26.

4 Munoz N, Mendez F, Posso H, et al. Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results. *J Infect Dis* 2004; **15:** 2077–87.

5 de Sanjose S, Diaz M, Castellsagué X, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect Dis* 2007; **7:** 453–59.

6 Wellings K, Collumbien M, Slaymaker E, et al. Sexual behaviour in context: a global perspective. *Lancet* 2006; **368:** 1706–28.

7 Betsey Stevenson, Justin Wolfers. Marriage and divorce: changes and their driving forces. *J Econ Pers* 2006; **21:** 27–52.

8 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.

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

10 The FUTURE II Study Group. Prophylactic efficacy of a quadrivalent human papillomavirus (HPV) vaccine in women with virologic evidence of HPV infection. *J Infect Dis* 2007; **196:** 1438–46.

11 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* 2007; **25:** 4931–39.

12 Insinga RP, Dasbach EJ, Elbasha EH, Liaw KL, Barr E. Progression and regression of incident cervical HPV 6, 11, 16 and 18 infections in young women. *Infect Agent Cancer* 2007; **2:** 15.

13 Muñoz N, Castellsague X, de Gonzalez AB, Gissmann L. HPV in the etiology of human cancer. *Vaccine* 2006; **24:** S1–10.

14 Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda system: terminology for reporting results of cervical cytology. *JAMA* 2002; **287:** 2114–19.

15 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.

16 WHO. Human papillomavirus infection and cervical cancer. Geneva: World Health Organization, 2004. http://www.who.int/vaccine_research/diseases/hpv/en/ (accessed July 5, 2007).

17 Pagliusi SR, Aguado TA. Efficacy and other milestones for human papillomavirus vaccine introduction. *Vaccine* 2004; **23:** 569–78.

18 Hildesheim A, Herrero R, Wacholder S, et al. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine among young women with preexisting infection: a randomized trial. *JAMA* 2007; **298:** 743–53.