

Evaluation of quadrivalent HPV 6/11/16/18 vaccine efficacy against cervical and anogenital disease in subjects with serological evidence of prior vaccine type HPV infection

Sven-Eric Olsson,^{1*} Susanne K. Kjaer,² Kristján Sigurdsson,³ Ole-Erik Iversen,⁴ Mauricio Hernandez-Avila,⁵ Cosette M. Wheeler,⁶ Gonzalo Perez,^{7,27} Darron R. Brown,⁸ Laura A. Koutsky,⁹ Eng Hseon Tay,¹⁰ Patricia García,¹¹ Kevin A. Ault,¹² Suzanne M. Garland,¹³ Sepp Leodolter,¹⁴ Grace W.K. Tang,¹⁵ Daron G. Ferris,¹⁶ Jorma Paavonen,¹⁷ Matti Lehtinen,¹⁸ Marc Steben,¹⁹ F. Xavier Bosch,²⁰ Joakim Dillner,²¹ Elmar A. Joura,¹³ Slawomir Majewski,²² Nubia Muñoz,²³ Evan R. Myers,²⁴ Luisa L. Villa,²⁵ Frank J. Taddeo,²⁶ Christine Roberts,²⁶ Amha Tadesse,²⁶ Janine Bryan,²⁶ Roger Maansson,²⁶ Scott Vuocolo,²⁶ Teresa M. Hesley,²⁶ Alfred Saah,²⁶ Eliav Barr²⁶ and Richard M. Haupt²⁶

¹Karolinska Institute at Danderyd Hospital; Stockholm, Sweden; ²Department of Virus, Hormones and Cancer; Institute of Cancer Epidemiology; Danish Cancer Society/Rigshospitalet; University of Copenhagen; Denmark; ³National Cancer Detection Clinic; Reykjavik, Iceland; ⁴Department of Clinical Medicine; University of Bergen; and Department of Obstetrics and Gynecology; Haukeland University Hospital; Bergen, Norway; ⁵Institute of Public Health; Cuernavaca, Morelos Mexico; ⁶Departments of Molecular Genetics and Microbiology and Obstetrics and Gynecology; University of New Mexico; Albuquerque, NM USA; ⁷Universidad del Rosario; Bogotá, Colombia; ⁸Department of Medicine; Indiana University School of Medicine; Indianapolis, IN USA; ⁹Department of Epidemiology; University of Washington; Seattle, WA USA; ¹⁰KK Women's & Children's Hospital; Singapore, Singapore; ¹¹Epidemiology HIV and STD Unit; Universidad Peruana Cayetano Heredia; Lima, Peru; ¹²Department of Gynecology and Obstetrics; Emory University School of Medicine; Atlanta, GA USA; ¹³Microbiology and Infectious Diseases Department; Royal Women's Hospital and Department of Obstetrics and Gynecology; University of Melbourne; Melbourne, VIC Australia; ¹⁴Department of Gynecology and Obstetrics; Medical University of Vienna; Vienna, Austria; ¹⁵Department of Obstetrics and Gynecology; University of Hong Kong; HKSAR; ¹⁶Department of Family Medicine and Obstetrics and Gynecology; Medical College of Georgia; Augusta, GA USA; ¹⁷Department of Obstetric and Gynecology; University Central Hospital; Helsinki, Finland; ¹⁸School of Public Health; University of Tampere; Tampere, Finland; ¹⁹Direction Risques Biologiques; Environnementaux et Occupationnels; Institut National de Santé Publique du Québec; Montréal, QC Canada; ²⁰Institut Catala d'Oncologia; IDIBELL; Barcelona, Spain; ²¹Dept. of Medical Microbiology; Lund University; Sweden; ²²Department of Dermatology and Venereology; Center of Diagnostics and Treatment of Sexually Transmitted Diseases; Warsaw Medical University; Warsaw, Poland; ²³National Institute of Cancer; Bogotá, Colombia; ²⁴Department of Obstetrics and Gynecology; Duke University Medical Center; Durham, NC USA; ²⁵Department of Virology; Ludwig Institute for Cancer Research; Sao Paulo, Brazil; ²⁶Merck Research Laboratories; West Point, PA USA; ²⁷current affiliation MSD Farmaceutica Ltd., Sao Paulo, Brazil

Keywords: HPV, cervical cancer, vaccine

Objective: In the quadrivalent (types 6/11/16/18) HPV vaccine (GARDASIL®/SILGARD®) clinical program, 73% of women aged 16–26 were naïve to all vaccine HPV types. In these women, prophylactic administration of the vaccine was highly effective in preventing HPV 6/11/16/18-related cervical disease. Of the remaining women, 15% of had evidence of past infection with one or more vaccine HPV types (seropositive and DNA negative) at the time of enrollment. Here we present an analysis in this group of women to determine the efficacy of the HPV 6/11/16/18 vaccine against new cervical and external anogenital disease related to the same vaccine HPV type which had previously been cleared. Vaccine tolerability in this previously infected population was also assessed.

Results: Subjects were followed for an average of 40 months. Seven subjects in the placebo group developed cervical disease, and eight subjects developed external genital disease related to a vaccine HPV type they had previously encountered. No subject receiving HPV 6/11/16/18 vaccine developed disease to a vaccine HPV type to which they were seropositive and DNA negative at enrollment.

Methods: 18,174 women were enrolled into 3 clinical studies. The data presented comprise a subset of these subjects (n = 2,617) who were HPV seropositive and DNA negative at enrollment (for ≥1 vaccine type). In each study, subjects were randomized in a 1:1 ratio to receive HPV 6/11/16/18 vaccine or placebo at day 1, month 2 and month 6 (without knowledge of baseline HPV status). Procedures performed for efficacy data evaluation included detailed genital examination, Pap testing and collection of cervicovaginal and external genital specimens. Analyses of efficacy were carried out in a population stratified by HPV serology and HPV DNA status at enrollment.

Conclusions: These results suggest that natural HPV infection-elicited antibodies may not provide complete protection over time, however the immune response to the HPV 6/11/16/18 vaccine appears to prevent reinfection or reactivation of disease with vaccine HPV types. Vaccine-related adverse experiences were higher among subjects receiving vaccine, mostly due to increased injection site adverse experiences.

*Correspondence to: Sven-Eric Olsson; Email: sven-eric.olsson@ds.se

Submitted: 04/22/09; Revised: 06/26/09; Accepted: 07/14/09

Previously published online: www.landesbioscience.com/journals/vaccines/article/9515

Introduction

Cervical cancer is the second leading cause of death attributable to cancer among women worldwide, and more than 99% of all cervical cancers contain HPV DNA.¹ The well-established causal link between HPV and cervical cancer and the high prevalence of HPV infection has led to the development of prophylactic vaccines directed against the most common, high-risk oncogenic HPV types.² Data suggest that within 3 years after initiation of sexual activity, up to 48% of women will have evidence of cervical human papillomavirus (HPV) infection.³

Phase III trials conducted in approximately 18,150 young adult women have demonstrated that a prophylactic quadrivalent (types 6/11/16/18) HPV L1 virus-like particle (VLP) vaccine was highly effective in preventing HPV 6-, 11-, 16- or 18-related cervical, vaginal and vulvar neoplasias (as well as anogenital condylomata) and persistent infection in women who were naïve to the respective vaccine HPV types at enrollment.^{4,5} Additionally, a bivalent HPV 16/18 VLP vaccine was shown to be effective in preventing persistent HPV16/18 infection among women naïve to HPV 16/18 prior to vaccination.⁶ However, the ability of prophylactic HPV vaccines to attenuate or abrogate disease in women who have persistent antibodies from a previous infection with a vaccine HPV type has not yet been defined.

This report presents the results of an analysis of data from three randomized, double-blind, placebo-controlled clinical trials to investigate the prophylactic efficacy of the quadrivalent HPV vaccine against disease related to HPV 6, 11, 16 or 18 in subjects who have previously been infected with ≥ 1 vaccine HPV type. This analysis was conducted to determine whether subjects with serological evidence of past HPV 6, 11, 16 or 18 infection, but with no evidence of current HPV 6, 11, 16 or 18 cervical/anogenital infection (with the same HPV type) benefit from vaccination with quadrivalent HPV vaccine. These data will also address the tolerability of vaccination in subjects who have previously been exposed to a vaccine HPV type, and remain seropositive to that type.

Results

Baseline demographic characteristics between subjects HPV PCR negative and seropositive for HPV 6/11/16/18 and the overall trial population can be seen in **Table 2**. In general, subjects seropositive at baseline had a higher mean number of sexual partners. These subjects also had a higher incidence of past pregnancy, and a history of chlamydia infection roughly double that of the overall population. Seropositive subjects were also more likely to be diagnosed with low-grade squamous intraepithelial lesions (LSIL) at enrollment.

In the overall population, 8.1%, 2.0%, 11.3% and 3.7% of subjects were seropositive to HPV 6, 11, 16 and 18 at enrollment, respectively (**Table 3**) (without respect to HPV DNA status). The majority of subjects who had antibodies to a specific vaccine HPV type at baseline were DNA negative to that HPV type as determined by PCR analysis, though a higher percentage of HPV 16 DNA positivity was seen in comparison to the other HPV types. In total, 6.4%, 1.8%, 6.9% and 2.7% of subjects were seropositive and PCR negative to HPV 6, 11, 16 and 18,

respectively. The proportion of seropositive and PCR negative women was balanced between vaccine and placebo groups for all vaccine HPV types.

Vaccine efficacy against HPV 6/11/16/18-related CIN 1 or worse in subjects seropositive and DNA negative to the relevant HPV type at baseline was 100% (95% CI: 28.7, 100) (**Table 4**). No vaccinated subjects developed cervical disease due to an HPV type with which they had previously been infected. Seven subjects receiving placebo developed cervical disease due to one of these types. Six of these cases were related to HPV 16 and one was related to HPV 18. Efficacy against the incidence of HPV 6/11/16/18-related external genital lesions in subjects seropositive and DNA negative to the relevant HPV type at baseline was also 100% (95% CI: 39.5, 100) (**Table 5**). Again, no cases were seen amongst vaccinated subjects, while there were eight placebo subjects who developed external genital disease related to a vaccine HPV type with which they had previously been infected. Five of these cases were related to HPV 6, two were related to HPV 16, and one related to HPV 18. Details of women diagnosed with CIN or EGL can be seen in **Figure 1**.

Subjects in the detailed safety population ($n = 948$) (filled out vaccine report cards) given vaccine reported slightly more adverse experiences when compared to those given placebo (92.5% vaccine versus 85.8% placebo) (**Table 6**). This difference was due predominantly to injection-site adverse experiences (84.1% vaccine versus 75.3% placebo). Serious vaccine-related adverse experiences included gastroenteritis, headache and hypertension (in the total safety population), and bronchospasm (in the detailed safety population).

Discussion

In the current analysis, we examined the data from a population of subjects who were previously infected with a vaccine HPV type (seropositive and DNA negative at baseline) prior to enrolling in a quadrivalent HPV vaccine clinical trial. Subjects were followed for an average of 40 months. Seven subjects in the placebo group developed cervical disease related to a vaccine HPV type they had previously encountered. Out of these seven vaccine related cases, six were due to HPV 16, 1 was due to HPV 18. Non-vaccine high-risk HPV types were found in 2 cases of HPV 16 related disease (16 + 58; 16 + 33) and one case of HPV 18 related disease (18 + 33 + 52 + 56). Eight subjects in the placebo group developed HPV-related external anogenital lesions, (Condyloma, VIN or VaIN) related to a vaccine HPV type they had previously encountered. Out of these eight EGLs, five were due to HPV 6, two were due to HPV 16 and 1 was due to HPV 18. No non-vaccine types (out of 10 tested types) were found. No subject receiving HPV 6/11/16/18 vaccine developed cervical or external genital disease related to a vaccine HPV type to which they were seropositive and DNA negative at enrollment (vaccine efficacy: 100% [cervical 95% CI: 28.7, 100.0; external anogenital 95% CI: 39.5, 100.0]).

As a means of prophylaxis, vaccine utility is greatest when given to those individuals who are susceptible to the relevant pathogen(s). In the case of HPV, this would entail immunization before sexual debut, when both girls and boys are not likely to

have been previously exposed to HPV. While subjects in the current analysis were still susceptible to vaccine HPV types, the overall vaccine effect (as measured by rate reductions) is smaller in the current analysis when compared to previous analyses of subjects who were PCR and seronegative to vaccine HPV types at baseline. This is possibly due to the protective effect of acquired immunity in the seropositive subjects. This prior seropositivity would have led to a humoral response, and potentially had a better chance of preventing the development of a lesion when compared to subjects with no (or undetectable levels) antibodies before infection.

Literature suggests that within 4 years after sexual debut, up to 63% of women may be infected with any HPV type.³ Data such as these highlight the importance of early intervention with respect to prevention of cervical cancer. However, regardless of efficacy or effectiveness, HPV vaccine implementation strategies are also likely to include some women who have already been infected. Detection of early HPV infections on internal mucosal epithelial sites is difficult, as there are few clinical indications that infection has occurred. Early detection relies on HPV DNA testing of genital specimens or the Pap test to detect abnormal cells resulting from HPV infection. Determining the HPV status of women prior to vaccination would require commercial availability of standardized and validated assays for detection of HPV type-specific antibodies. Although such assays are not currently available they would likely be costly and may be difficult to implement from a global perspective, thus information concerning the efficacy of the vaccine in women with prior exposure to HPV (though not currently infected) is reassuring.

It is well known that HPV does not always elicit a measurable immune response, due largely in part to the non-lytic nature of HPV viral replication.⁷ An example of this in the current study is the observation that infection-elicited immune responses against HPV were not always sufficient to prevent subjects in the placebo group from developing subsequent disease related to that HPV type, even though they may have eradicated the virus upon initial exposure. There is debate about why some subjects develop disease upon re-exposure to HPV and some do not. This debate primarily stems from the uncertainty surrounding the legitimacy of the “reinfection” concept. The alternative explanation being that some people may have low-level persistent HPV infections (also known as latency) that are able to remain undetected due to issues of host immune competence.⁸ Therefore, in these subjects, apparent reinfection could be a re-emergence of a low-level persistent infection that was never totally eradicated. HPV infection of mucosal surfaces are typically asymptomatic, and present few, if any clinical signs. Further study is required to delineate these two possibilities.

Concerns over the tolerability of receiving the quadrivalent HPV vaccine when a subject already had antibodies for vaccine HPV type/s have been raised. As can be seen from these results, vaccine-related adverse experiences are higher in those receiving HPV vaccine, mostly related to increased injection-site related adverse experiences. These results are however similar to data seen in per-protocol subjects who were HPV naïve (vaccine HPV types) at enrollment,⁹ suggesting that a history of HPV-infection did not contribute to a demonstrably higher frequency of adverse events seen in the vaccine group in response to vaccination.

Identified limitations of this combined analysis are the lack of long-term follow-up and the relatively small number of women who were eligible for this analysis (PCR negative and seropositive for a vaccine HPV type). While these results should be considered preliminary, we may expect similar findings in larger studies with longer follow-up. Additionally, while associations between a specific vaccine HPV type and cervical or external anogenital disease were made based on finding HPV DNA in the lesion, the role of coinfecting HPV types must be considered. True causality for vaccine HPV types is not certain when there are HPV coinfections present.

In conclusion, these results suggest that antibodies elicited by natural infection may not provide complete protection from subsequent HPV reinfection/reactivation and related disease over time, and that the robust immune response to the quadrivalent HPV vaccine may prevent recurrence or reactivation of disease with vaccine HPV types. Further study will be needed to fully understand the kinetics of infection and disease in subjects who have been previously infected and who have apparently eradicated a vaccine HPV type.

Methods

Data sources. This report is derived from combined data gathered from 3 clinical trials (protocols 007, 013 and 015). Protocol 007 was a Phase IIb, randomized, multi-center, double-blind, placebo-controlled study. It was designed to facilitate the selection one of three formulations of quadrivalent HPV (types 6/11/16/18) L1 VLP vaccine (GARDASIL®/SILGARD®) for use in Phase III studies and to preliminarily evaluate the safety, immunogenicity and efficacy of the selected formulation.¹⁰ Data analyzed here from protocol 007 consist of the placebo and the HPV 6/11/16/18 vaccine formulation groups that were selected for use in phase III studies. Protocols 013 and 015 were Phase III, randomized, double-blind, placebo-controlled studies designed to investigate the effect of the quadrivalent HPV vaccine on disease caused by HPV 6, 11, 16 and 18.^{4,5} Subjects were followed for an average of 40 months.

Subjects. Female adolescents and young adults aged 16–26 were randomized into one of three clinical trials (protocols 007[n = 552], 013[n = 5,455] and 015[n = 12,167]). Participants enrolled were to be non-pregnant with no prior abnormal Papanicolaou smears. All subjects enrolled (18 years or older) were to have a lifetime history of four or fewer lifetime sex partners (a proportion of 16–17 year old Finnish girls had more than 4 lifetime sexual partners). Subjects with prior confirmed (via histology) HPV disease were excluded from enrolling; however those with prior or current HPV infection (determined by serology and PCR testing, respectively) were not excluded (including women with abnormal cytology). Enrolled subjects with clinical evidence of external anogenital HPV disease at day 1 were discontinued from the study prior to randomization. All participants or parents/legal guardians signed informed consents following review of the protocol procedures. Participants received intramuscular injections of the quadrivalent HPV vaccine or visually indistinguishable placebo at enrollment (day 1), month 2 and month 6. The studies were conducted in conformance with applicable national or local

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

Design feature	Protocol 007	Protocol 013	Protocol 015
General			
Sample size	552	5,455	12,167
Study dates	2000 to 2004	2001 to 2007	2002 to 2007
Study sites	International, Multicenter		
Study design	Prospective, parallel		
Blinding	Double-blind		
Vaccination regimen	0, 2, 6 months		
Visit schedule	Months 0, 7, 12, 18, 24, 30 and 36	Months 0, 3, 7, 12, 18, 24, 30, 36 and 48	Months 0, 7, 12, 24, 36 and 48
Inclusion/Exclusion criteria			
Age	16 to 23 years	16 to 23 years	15 to 26 years
Lifetime number of male sexual partners	0 to 4		
Previous known abnormal Pap	Not Allowed, but no exclusion if no prior test was available		
Previous known HPV disease	Not Allowed, but no exclusion of basis of ongoing infection or disease (i.e., CIN or abnormal cervical cytology at enrollment)		
Cervical cancer screening			
Timing of Pap test screening	Approximately every 6 months	Approximately every 6 months	Approximately every 12 months
Screening triage strategy	Voluntary	Mandatory	Mandatory
Minimal Pap abnormality for referral	ASCUS, HPV (+) on HC-II		

PCR, Polymerase Chain Reaction; ASCUS, Atypical Squamous Cells of Undetermined Significance; HPV, human papillomavirus; HC-II, Hybrid Capture II.

requirements regarding ethical committee review, informed consent and the protection of the rights and welfare of human subjects participating in biomedical research. A detailed description of the methodologies for each protocol included in the present analyses have been published previously.^{4,5,11}

Vaccine. The quadrivalent vaccine consisted of a mixture of four recombinant HPV type-specific VLPs composed of the L1 major capsid proteins of HPV types 6, 11, 16 and 18 produced in *Saccharomyces cerevisiae*.¹²⁻¹⁴ The vaccine is comprised of 20 ug of HPV 6 VLP, 40 ug of HPV 11 VLP, 40 ug of HPV 16 VLP and 20 ug of HPV 18 VLP, in a total carrier volume of 0.5 mL. The four VLP types were purified and adsorbed onto amorphous aluminum hydroxyphosphate sulfate adjuvant. The placebo contained the same adjuvant alone and was visually indistinguishable from vaccine.

Clinical follow-up. A complete gynecological history and a gynecological physical examination were conducted at enrollment and at specified time intervals thereafter for each individual clinical study (Table 1).^{4,5,10} ThinPrep™ (Cytoc, Boxborough MA, USA) cytology specimens for Papanicolaou (Pap) testing were collected at enrollment (day 1), month 7, and at regular intervals thereafter (Table 1). A urine pregnancy test was performed immediately prior to each vaccination. Subjects with a positive pregnancy test were not vaccinated. Participants were observed for 30 minutes after each injection and asked to report serious adverse experiences. A subset of subjects was directed to use a vaccination report card to capture all adverse experiences occurring (detailed safety cohort). Throughout the trial, all serious adverse experiences that were potentially procedure or vaccine-related and all deaths were also to be reported.

Cytology specimens were evaluated using the Bethesda System-2001. For cytology diagnoses of atypical squamous cells

of undetermined significance (ASC-US) or worse the laboratory automatically performed reflex HPV testing on residual ThinPrep™ material, using the Digene Hybrid Capture II™, High-Risk/Low-Risk Probes (Digene, Gaithersburg, MD, USA). Procedures for algorithm-based colposcopy and biopsy referral have been described previously.^{4,5} All biopsies were processed independently to avoid HPV DNA contamination. Biopsy samples were processed and read by a central laboratory (Diagnostic Cytology Laboratories, Indianapolis, IN, USA) for medical management. As described previously, endpoint assignment was done by use of consensus diagnoses from a panel of pathologists who were blinded to the central laboratory diagnoses, treatment group and HPV status.^{4,5} Subjects whose histological specimens were diagnosed as neoplasia were referred for definitive therapy according to mandatory protocol-prescribed guidelines.

For each subject, blood samples were obtained at enrollment and at defined intervals throughout the study for anti-HPV serology testing. Serum concentrations of antibodies to HPV 6, 11, 16 and 18 were measured by competitive immunoassay (Luminex Corporation, Austin, TX, USA). Dilution-corrected serostatus cutoffs were 20 mMU/mL for HPV 6, 16 mMU/mL for HPV 11, 20 mMU/mL for HPV 16 and 24 mMU/mL for HPV 18.

Endpoints and ascertainment of lesions for this analysis. Endpoint assignment in protocols 007, 013 and 015 was performed by a pathology panel blinded to central laboratory clinical diagnoses. A case was defined as a pathology panel consensus diagnosis of CIN or EGL with HPV 6, 11, 16 or 18 DNA detected in an adjacent section of the same lesion, in frozen tissue adjacent to the lesion (007 only), or in a swab of the lesion (007 only), and in cervicovaginal samples obtained at the visit antecedent to the biopsy visit, with the latter condition not required if vaccine

Table 2. Baseline demographics

	Vaccine (PCR negative, seropositive)*	Placebo (PCR negative, seropositive)†	Overall population	Overall population
	(N = 1,298)	(N = 1,319)	(N = 9,087)	(N = 9,087)
	n (%)	n (%)	n (%)	n (%)
Mean age ± SD	20.7 ± 1.8	20.6 ± 1.9	20.0 ± 2	20.0 ± 2
Among non-virgins				
Sexual debut (years)	16.4 ± 1.8	16.3 ± 1.8	16.7 ± 1.9	16.7 ± 1.9
Mean lifetime number of sexual partners				
Unknown	-	-	-	3 (0.0)
1	276 (21.4)	298 (22.7)	2,929 (34.2)	2,938 (34.5)
2	356 (27.6)	302 (23.0)	2,210 (25.8)	2,224 (26.1)
3	327 (25.3)	350 (26.7)	1,798 (21.0)	1,754 (20.6)
4	315 (24.4)	340 (25.9)	1,519 (17.7)	1,477 (17.3)
>4	18 (1.4)	21 (1.6)	111 (1.3)	128 (1.5)
Median	3	3	2	2
Past pregnancy	509 (39.2)	516 (39.1)	2,057 (22.6)	2,041 (22.5)
Using hormonal contraception	759 (58.5)	808 (61.3)	5,346 (58.9)	5,314 (58.5)
History of chlamydia	144 (11.1)	148 (11.2)	523 (5.8)	510 (5.6)
Pap test results				
LSIL	108 (8.5)	109 (8.5)	524 (5.9)	503 (5.7)
HSIL	13 (1.0)	11 (0.9)	62 (0.7)	47 (0.5)
ASC-US	83 (6.6)	98 (7.7)	398 (4.5)	421 (4.8)
ASC-H	5 (0.4)	6 (0.5)	28 (0.3)	25 (0.3)
Atypical glandular cells	-	-	6 (0.1)	3 (0.0)

PCR, polymerase chain reaction; SD, standard deviation; LSIL, low-grade squamous epithelial lesion; HSIL, high-grade squamous epithelial lesion; ASC-US, abnormal squamous cells of undetermined significance; ASC-H, atypical squamous cells-cannot exclude HSIL. N = subjects randomized; n = subjects with evaluable data. *Includes 1 subject who was seropositive to HPV type 6 at day 1; †excludes 1 subject who was seronegative to all vaccine HPV types at day 1.

Table 3. HPV 6, 11, 16 and 18 PCR status by serostatus at enrollment by vaccination group

	Vaccine†			Placebo†			Overall population†		
	Day 1 serostatus			Day 1 serostatus			Day 1 serostatus		
	n	Positive m (%)	Negative m (%)	n	Positive m (%)	Negative m (%)	n	Positive m (%)	Negative m (%)
HPV 6	9,056	733 (8.1)	8,323 (91.9)*	9,063	742 (8.2)	8,321 (91.8)	18,119	1,475 (8.1)	16,624 (91.9)
PCR positive		150 (1.7)	224 (2.5)		137 (1.5)	217 (2.4)		287 (1.6)	441 (2.4)
PCR negative		570 (6.3)	8,000 (88.3)		595 (6.6)	8,022 (88.5)		1,165 (6.4)	16,022 (88.4)
HPV 11	9,055	184 (2.0)	8,871 (98.0)	9,063	187 (2.1)	8,876 (97.9)	18,118	371 (2.0)	17,747 (98.0)
PCR positive		23 (0.3)	42 (0.5)		22 (0.2)	35 (0.4)		45 (0.2)	77 (0.4)
PCR negative		160 (1.8)	8,730 (96.4)		165 (1.8)	8,761 (96.7)		325 (1.8)	17,491 (96.5)
HPV 16	9,056	1,002 (11.1)	8,054 (88.9)	9,063	1,039 (11.5)	8,024 (88.5)†	18,119	2,041 (11.3)	16,078 (88.7)
PCR positive		371 (4.1)	431 (4.8)		389 (4.3)	407 (4.5)		760 (4.2)	837 (4.6)
PCR negative		610 (6.7)	7,522 (83.1)		639 (7.1)	7,534 (83.1)		1,249 (6.9)	15,056 (83.1)
HPV 18	9,055	330 (3.6)	8,725 (96.4)	9,063	338 (3.7)	8,725 (96.3)	18,118	668 (3.7)	17,450 (96.3)
PCR positive		78 (0.9)	242 (2.7)		95 (1.0)	233 (2.6)		173 (1.0)	475 (2.6)
PCR negative		246 (2.7)	8,383 (92.6)		239 (2.6)	8,413 (92.8)		485 (2.7)	16,796 (92.7)

Percentages are calculated as 100*(m/n). n = Number of randomized subjects with non-missing day 1 HPV cLIA results; m = Number of subjects who are serostatus positive (or negative) at enrollment; PCR = polymerase chain reaction. *Includes 1 subject who was seropositive to HPV type 6 at day 1; †excludes 1 subject who was seronegative to all vaccine HPV types at day 1; ‡Subjects with missing or unknown PCR and/or serology data are not included in the positive and negative breakdown.

Table 4. Efficacy against HPV 6/11/16/18-related CIN I or worse (seropositive, DNA negative subjects)

	Vaccine			Placebo			Efficacy (%)	95% CI
	n	Cases	Rate	n	Cases	Rate		
HPV 6/11/16/18	1,243	0	0.0	1,283	7	0.2	100.0	(28.7, 100.0)
by Severity*								
CIN I	1,243	0	0.0	1,283	6	0.1	100.0	(12.7, 100.0)
CIN 2 or Worse	1,243	0	0.0	1,283	4	0.1	100.0	(<0, 100.0)
CIN 2	1,243	0	0.0	1,283	1	0.0	100.0	(<0, 100.0)
CIN 3 or Worse	1,243	0	0.0	1,283	4	0.1	100.0	(<0, 100.0)
CIN 3	1,243	0	0.0	1,283	3	0.1	100.0	(<0, 100.0)
AIS	1,243	0	0.0	1,283	1	0.0	100.0	(<0, 100.0)
by HPV type*								
HPV 6	538	0	0.0	568	0	0.0	N/A	N/A
HPV 11	140	0	0.0	146	0	0.0	N/A	N/A
HPV 16	574	0	0.0	625	6	0.3	100.0	(8.5, 100.0)
HPV 18	236	0	0.0	233	1	0.1	100.0	(<0, 100.0)

CI, confidence interval; rate = incidence rate per 100 person years; CIN, cervical intraepithelial neoplasia; AIS, adenocarcinoma in situ; n = number of subjects evaluable, i.e., number of subjects in the given population who also have at least one follow-up visit 30 days after day 1. *A subject is counted once in each applicable endpoint category—a subject may appear in more than one category.

Table 5. Efficacy against HPV 6/11/16/18-related external genital lesions (seropositive, DNA negative subjects)

	Vaccine			Placebo			Efficacy (%)	95% CI
	n	Cases	Rate	n	Cases	Rate		
HPV 6/11/16/18	1,268	0	0.0	1,301	8	0.2	100%	(39.5, 100.0)
by Severity*								
Condyloma	1,268	0	0.0	1,301	7	0.2	100.0	(28.3, 100.0)
VIN I or VaIN I	1,268	0	0.0	1,301	1	0.0	100.0	(<0, 100.0)
VIN2/3 or VaIN2/3	1,268	0	0.0	1,301	2	0.0	100.0	(<0, 100.0)
by HPV type*								
HPV 6	546	0	0.0	579	5	0.3	100.0	(<0, 100.0)
HPV 11	142	0	0.0	149	0	0.0	N/A	N/A
HPV 16	587	0	0.0	632	2	0.0	100.0	(<0, 100.0)
HPV 18	241	0	0.0	234	1	0.1	100.0	(<0, 100.0)

CI, confidence interval; rate = incidence rate per 100 person years; CIN, cervical intraepithelial neoplasia; AIS = adenocarcinoma in situ; n = number of subjects evaluable, i.e., number of subjects in the given population who also have at least one follow-up visit 30 days after day 1. *A subject is counted once in each applicable endpoint category—a subject may appear in more than one category.

HPV type DNA was detected in an adjacent section of the same lesion. DNA detection was determined by multiplex PCR assay developed by Merck Research Laboratories. To qualify as an endpoint related to a vaccine HPV type, the subject was also required to be seropositive and DNA negative to the same HPV type at baseline.

Colposcopy referral was standardized using a mandatory Papanicolaou triage algorithm. Colposcopists were trained to locate and biopsy all discrete abnormal areas on the cervix. Separate instruments were used to avoid HPV contamination. Biopsy samples were processed and adjacent histologic sections of each biopsy were first read for clinical management by pathologists at a central laboratory (Diagnostic Cytology Laboratories, Indianapolis, IN) and then read for endpoint determination by a panel of at least two and up to four pathologists who were blinded to central laboratory clinical diagnoses, treatment group and HPV

status. Subjects with cervical intraepithelial neoplasia grade 2/3 or worse were referred for definitive therapy. Definitive therapy was performed as clinically indicated.

Statistical methods. In this analysis, subjects were required to have received ≥ 1 vaccination and be seropositive and PCR negative to ≥ 1 vaccine HPV type(s) at day 1 to be eligible for the analysis of that HPV type. Protocol violators were included. Case counting began after day 30.

A point estimate of vaccine efficacy and the 95% percent confidence interval (CI) were calculated on the basis of the observed case split between vaccine and placebo recipients and the accrued person-time. An exact conditional procedure was used to evaluate vaccine efficacy under the assumption that the numbers of cases in the vaccine and placebo groups were independent Poisson random variables. If a subject developed more than one endpoint, she was counted as a case at the date of the first endpoint.

Table 6. Clinical adverse experience summary

	Vaccine		Placebo	
	(N = 1259)		(N = 1279)	
	n	(%)	n	(%)
Total safety population*				
Subjects in analysis population	1259		1279	
Subjects with follow-up	1233		1264	
Number (%) of subjects:				
with one or more adverse experiences	486	(39.4)	437	(34.6)
injection-site adverse experiences	423	(34.3)	364	(28.8)
systemic adverse experiences	312	(25.3)	298	(23.6)
with vaccine-related [†] adverse experiences	461	(37.4)	402	(31.8)
injection-site adverse experiences	422	(34.2)	363	(28.7)
systemic adverse experiences	227	(18.4)	187	(14.8)
with serious adverse experiences	11	(0.9)	8	(0.6)
with serious vaccine-related adverse experiences	3	(0.2)	0	(0.0)
who died	2	(0.2)	0	(0.0)
discontinued [‡] due to an adverse experience	5	(0.4)	0	(0.0)
discontinued due to a serious adverse experience	2	(0.2)	0	(0.0)
discontinued due to a vaccine-related adverse experience	2	(0.2)	0	(0.0)
discontinued due to a serious vaccine-related adverse experience	0	(0.0)	0	(0.0)
Detailed safety population**				
Subjects in analysis population	480		468	
Subjects with follow-up	465		458	
Number (%) of subjects:				
with one or more adverse experiences	430	(92.5)	393	(85.8)
injection-site adverse experiences	391	(84.1)	345	(75.3)
systemic adverse experiences	284	(61.1)	265	(57.9)
with vaccine-related [†] adverse experiences	418	(89.9)	372	(81.2)
injection-site adverse experiences	391	(84.1)	344	(75.1)
systemic adverse experiences	211	(45.4)	168	(36.7)
with serious adverse experiences	7	(1.5)	4	(0.9)
with serious vaccine-related adverse experiences	1	(0.2)	0	(0.0)
who died	1	(0.2)	0	(0.0)
discontinued [‡] due to an adverse experience	3	(0.6)	0	(0.0)
discontinued due to a serious adverse experience	1	(0.2)	0	(0.0)
discontinued due to a vaccine-related adverse experience	1	(0.2)	0	(0.0)
discontinued due to a serious vaccine-related adverse experience	0	(0.0)	0	(0.0)

*All subjects with safety data; **all subjects filling out vaccine report cards (VRC); [†]Determined by the investigator to be possibly, probably, or definitely related to the vaccine; [‡]Discontinued = subject discontinued from therapy. Percentages are calculated based on the number of subjects with follow-up.

Acknowledgements

We would like to thank Margaret James and Shuang Lu for help with statistical analyses, and Mary Anne Rutkowski and Kathy Harkins for help with statistical programming.

Financial disclosure

Source of Funding: Merck Research Laboratories, a Division of Merck & Company, Inc., funded these studies.

Role of the Funding Source: The studies were designed by the sponsor (Merck and Co., Inc.) in collaboration with external investigators and an external data and safety monitoring board.

The sponsor collected the data, monitored the conduct of the study, performed the statistical analysis and coordinated 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.

Conflict of interest

N.M. has received lecture fees, advisory board fees, and consultancy fees from Merck and Sanofi Pasteur MSD. S.E.O. has received lecture fees from Merck. M.H.A. has received lecture fees and grant

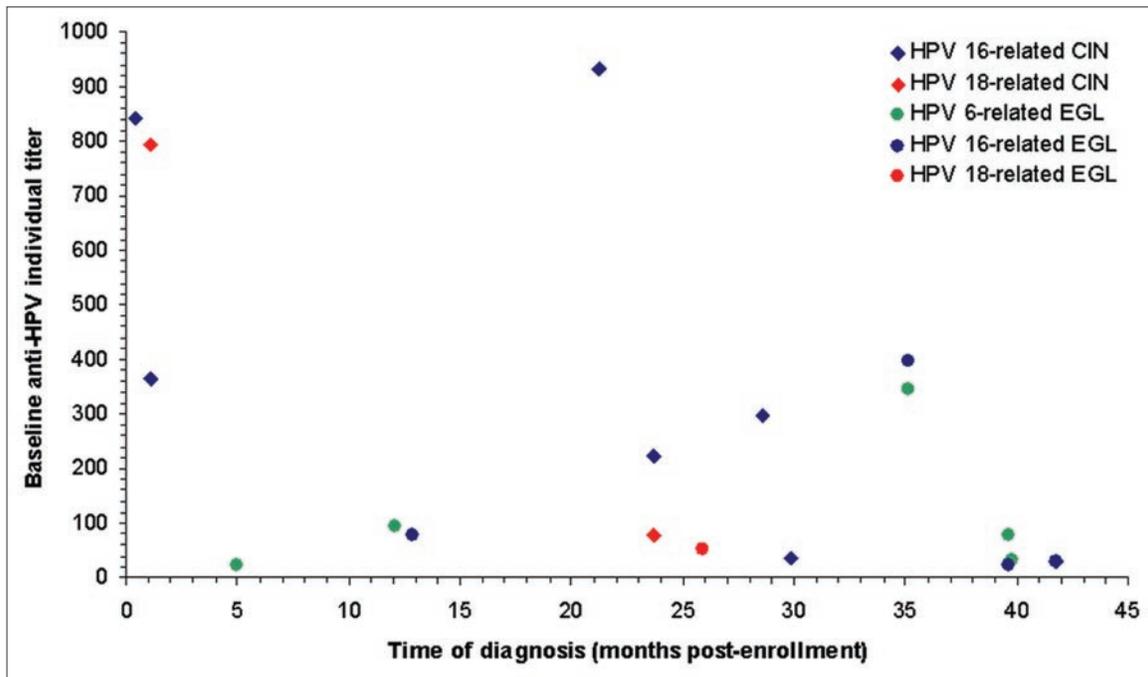


Figure 1. Temporal incidence of CIN and EGL (related to the HPV type women were seropositive and DNA negative to at enrollment) plotted against the woman's individual titer at baseline for the relevant HPV type. HPV 6, 11, 16 and 18 geometric mean titer at baseline for women seropositive to HPV 6, 11, 16 and 18 at baseline was 64.3 mMU/mL (95% CI: 60.3, 68.5), 40.2 mMU/mL (95% CI: 35.9, 45.0), 98.0 mMU/mL (95% CI: 91.8, 104.7) and 73.7 mMU/mL (95% CI: 67.0, 81.0), respectively. Titers for each type are determined relative to a type specific standard, and therefore titers were not equilibrated to allow from cross comparison.

support from Merck. O.E.I. has received lecture fees from Merck and GlaxoSmithKline. C.M.W. has received funding through her institution to conduct HPV vaccine studies for GlaxoSmithKline, and Roche Molecular Systems. K.A. has received consultancy and advisory board fees from Merck. X.B. has received lecture fees from Merck and GlaxoSmithKline, and has received funding through his institution to conduct HPV vaccine studies GlaxoSmithKline. J.P. has received consultancy fees, advisory board fees and lecture fees from Merck. J.D. has received consultancy fees, lecture fees and research grants from Merck and Sanofi Pasteur MSD. S.L. has received lecture fees from Merck and Sanofi Pasteur MSD. E.J. has received lecture fees from Merck, Sanofi Pasteur MSD and GlaxoSmithKline. S.K.K. has received consultancy fees, and has received funding through her institution to conduct HPV vaccine studies for Sanofi Pasteur MSD and Digene. S.M.G. has received advisory board fees and grant support from Commonwealth Serum

Laboratories and GlaxoSmithKline, and lecture fees from Merck. D.G.F. has received consultancy fees and funding through his institution to conduct HPV vaccine studies for GlaxoSmithKline and Merck, and lecture fees and consultancy fees from Merck. K.S. has received consultancy fees from Merck. S.M. has received lecture fees and advisory board fees from Merck. G.P. has received lecture fees and consultancy fees from Merck and Sanofi Pasteur MSD. D.R.B. has received lecture fees, advisory board fees, and intellectual property fees. M.S. has received lecture fees and grant support from Merck. Additionally, S.E.O., C.M.W., M.H.A., L.L.V., O.E.I., G.W.K.T., X.B., J.P., J.D., M.L., E.H.T., S.L., E.J., S.K.K., G.P., D.G.F., K.S., M.S., L.A.K. and D.R.B. have received funding through their institutions to conduct HPV vaccine studies for Merck. F.J.T., C.R., A.T., J.B., R.M., S.V., S.L., T.M.H., A.S., R.H. and E.B. are employees of Merck and potentially own stock and/or stock options in the company.

References

- Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999; 189:12-9.
- Christensen ND. Emerging human papillomavirus vaccines. *Expert Opin Emerg Drugs* 2005; 10:5-19.
- 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; 109:96-8.
- 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.
- The FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N Engl J Med* 2007; 356:1915-27.
- Harper DM, Franco EL, Wheeler CM, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection of human papillomavirus type 16 and 18 in young women: a randomised controlled trial. *Lancet* 2004; 364:1757-65.
- Stanley M. Immune responses to human papillomavirus. *Vaccine* 2006; 24:16-22.
- Doorbar J. Molecular biology of human papillomavirus infection and cervical cancer. *Clin Sci (Lond)* 2006; 110:525-41.
- Barr E, Tamms G. Quadrivalent human papillomavirus vaccine. *Clin Infect Dis* 2007; 45:609-17.
- 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 Oncology* 2005; 6:271-8.
- 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.
- Lowe RS, Brown DR, Bryan JT, et al. Human papillomavirus type 11 (HPV-11) neutralizing antibodies in the serum and genital mucosal secretions of African green monkeys immunized with HPV-11 virus-like particles expressed in yeast. *J Infect Dis* 1997; 176:1141-5.
- Koutsky LA, Ault KA, Wheeler CM, et al. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002; 347:1645-51.
- Ault K, Giuliano AR, Edwards R, et al. A phase I study to evaluate a human papillomavirus (HPV) type 18 L1 VLP vaccine. *Vaccine* 2004; 22:3004-7.