Efficacy, safety, and immunogenicity of the human papillomavirus 16/18 AS04-adjuvanted vaccine in women older than 25 years: 4-year interim follow-up of the phase 3, double-blind, randomised controlled VIVIANE study

S Rachel Skinner, Anne Szarewski, Barbara Romanowski, Suzanne M Garland, Eduardo Lazcano-Ponce, Jorge Salmerón, M Rowena Del Rosario-Raymundo, René H M Verheijen, Swee Chong Quek, Daniel P da Silva, Henry Kitchener, Kah Leng Fong, Céline Bouchard, Deborah M Money, Arunachalam Ilancheran, Margaret E Cruickshank, Myron J Levin, Archana Chatterjee, Jack T Stapleton, Mark Martens, Wim Quint, Marie-Pierre David, Dorothée Meric, Karin Hardt, Dominique Descamps, Brecht Geeraerts, Frank Struyf, Gary Dubin, for the VIVIANE Study Group*

Summary

Background Although adolescent girls are the main population for prophylactic human papillomavirus (HPV) vaccines, adult women who remain at risk of cervical cancer can also be vaccinated. We report data from the interim analysis of the ongoing VIVIANE study, the aim of which is to assess the efficacy, safety, and immunogenicity of the HPV 16/18 AS04-adjuvanted vaccine in adult women.

Methods In this phase 3, multinational, double-blind, randomised controlled trial, we randomly assigned healthy women older than 25 years to the HPV 16/18 vaccine or control (1:1), via an internet-based system with an algorithm process that accounted for region, age stratum, baseline HPV DNA status, HPV 16/18 serostatus, and cytology. Enrolment was age-stratified, with about 45% of participants in each of the 26–35 and 36–45 years age strata and 10% in the 46 years and older stratum. Up to 15% of women in each age stratum could have a history of HPV infection or disease. The primary endpoint was vaccine efficacy against 6-month persistent infection or cervical intraepithelial neoplasia grade 1 or higher (CIN1+) associated with HPV 16/18. The primary analysis was done in the according-to-protocol cohort for efficacy, which consists of women who received all three vaccine or control doses, had negative or low-grade cytology at baseline, and had no history of HPV disease. Secondary analyses included vaccine efficacy against non-vaccine oncogenic HPV types. Mean follow-up time was 40·3 months. This study is registered with ClinicalTrials.gov, number NCT00294047.

Findings The first participant was enrolled on Feb 16, 2006, and the last study visit for the present analysis took place on Dec 10, 2010; 5752 women were included in the total vaccinated cohort (n=2881 vaccine, n=2871 control), and 4505 in the according-to-protocol cohort for efficacy (n=2264 vaccine, n=2241 control). Vaccine efficacy against HPV 16/18-related 6-month persistent infection or CIN1+ was significant in all age groups combined (81·1%, 97·7% CI 52·1–94·0), in the 26–35 years age group (83·5%, 45·0–96·8), and in the 36–45 years age group (77·2%, 2·8–96·9); no cases were seen in women aged 46 years and older. Vaccine efficacy against atypical squamous cells of undetermined significance or greater associated with HPV 16/18 was also significant. We also noted significant cross-protective vaccine efficacy against 6-month persistent infection with HPV 31 (79·1%, 97·7% CI 27·6–95·9) and HPV 45 (76·9%, 18·5–95·6]) Serious adverse events occurred in 285 (10%) of 2881 women in the vaccine group and 267 (9%) of 2871 in the control group; five (<1%) and eight (<1%) of these events, respectively, were believed to be related to vaccination.

Interpretation In women older than 25 years, the HPV 16/18 vaccine is efficacious against infections and cervical abnormalities associated with the vaccine types, as well as infections with the non-vaccine HPV types 31 and 45.

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*Dr Szarewski died in August, 2013

Vaccines Trials Group, Telethon Institute for Child Health Research, Perth, WA, Australia (S R Skinner PhD); Sydney University Discipline of Paediatrics and Child Health, Children's Hospital Westmead, Sydney, NSW, Australia (S R Skinner); Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, London, UK (A Szarewski PhD); Division of Infectious Diseases, Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada (Prof B Romanowski MD); Department of Microbiology and Infectious Diseases, The Royal Women's Hospital, Parkville, VIC, Australia (Prof S M Garland FRCPA); Department of Microbiology, The Royal Children's Hospital, Parkville, VIC, Australia (S M Garland); Murdoch Childrens Research Institute, Parkville, VIC, Australia (S M Garland); Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, VIC, Australia (S M Garland); Avenida Universidad S/N Jojutla, Morelos, Mexico (Prof E Lazcano-Ponce PhD); Unidad de Investigación Epidemiológica y en Servicios de Salud, Instituto Mexicano del Seguro Social, Morelos, Mexico

Introduction

Infection with an oncogenic genotype of human papillomavirus (HPV) is a central precursor of cervical cancer,[1] with HPV types 16, 18, 45, 31, and 33 together accounting for about 85% of cases of invasive cervical cancer.[2] Although the risk of acquisition of HPV infection is greatest in young, sexually active women, women older than 25 years are also vulnerable to new infections.[3,4] Additionally, HPV infections can reappear after a period of non-detection.[5] The same and different HPV variants of a single genotype can be detected in the same individual over time, sometimes interspersed with negative findings.[6,7] This pattern is probably caused either by persistent infection that intermittently falls below the threshold for HPV DNA detection or the acquisition of new infection. Many HPV infections detected in adult women are likely to be new because they are associated with new sexual partners.[8]

(Prof J Salmerón PhD); **Department of Obstetrics and Gynecology, San Pablo Colleges Medical Center, San Pablo City, Laguna, Philippines** (M R Del Rosario-Raymundo MD); **Gynaecological Oncology and HumVac Research Group, University Medical Center Utrecht, Utrecht, Netherlands** (Prof R H M Verheijen MD); **Parkway Gynaecology Screening & Treatment Centre, Gleneagles Hospital, Singapore** (S C Quek MBBCh); **Departmento de Ginecologia, Instituto Português de Oncologia de Coimbra, Coimbra, Portugal** (D P da Silva MD); **Women's Cancer Centre, Institute of Cancer Sciences, University of Manchester, St Mary's Hospital, Manchester, UK** (Prof H Kitchener MD); **Department of Obstetrics and Gynaecology, Singapore General Hospital, Singapore** (K L Fong MRCOG); **Clinique RSF, Québec, QC, Canada** (C Bouchard FRCSC); **Department of Obstetrics and Gynaecology, University of British Columbia, Vancouver, BC, Canada** (Prof D M Money MD); **The Women's Health Research Institute, Vancouver, BC, Canada** (D M Money); **Division of Gynecologic Oncology, National University Hospital, Singapore** (A Ilancheran MD); **Division of Medical Education, University of Aberdeen, Aberdeen, UK** (Prof M E Cruickshank MD); **University of Colorado Denver, Anschutz Medical Campus, Aurora, CO, USA** (Prof M J Levin MD); **Department of Pediatrics, University of South Dakota Sanford School of Medicine, and Sanford Children's Specialty Clinics, Sioux Falls, SD, USA** (Prof A Chatterjee MD); **Department of Internal Medicine, University of Iowa, Iowa City, IA, USA** (Prof J T Stapleton MD); **Jersey Shore University Medical Center, Neptune, NJ, USA** (M Martens MD); **DDL Diagnostic Laboratory, Rijswijk, Netherlands** (W Quint PhD); **GSK Vaccines, Wavre, Belgium** (M-P David MSc, D Meric MSc, K Hardt PhD, D Descamps MD, B Geeraerts PhD, F Struyf MD); **and GSK Vaccines, King of Prussia, PA, USA** (G Dubin MD)

Correspondence to: S Rachel Skinner, Clinical School, Discipline of Paediatrics & Child Health, University of Sydney,

Two HPV vaccines are now licensed in many countries; the HPV 16/18 AS04-adjuvanted vaccine[9–11] and the HPV 6/11/16/18 vaccine.[12–14] The main population for prophylactic HPV vaccination is adolescent girls before sexual debut. Nevertheless, interest in the vaccine among adult women is strong,[15,16] especially among women who are aware of the risk of cervical cancer and attend screening regularly, and those who have had a previous abnormality. Currently, HPV vaccines are licensed for use in women up to age 45 years in some countries (such as Canada and Australia), and for older women in some others (such as Switzerland and the European Union). The efficacy of the HPV 6/11/16/18 vaccine in women aged 24–45 years has already been described.[17,18]

Here we report efficacy, safety, and immunogenicity data from the interim analysis of the ongoing Human PapillomaVIrus: Vaccine Immunogenicity ANd Efficacy (VIVIANE) study of the HPV 16/18 vaccine in women older than 25 years.

Methods

Study design and participants

VIVIANE is an ongoing phase 3, multinational, double-blind, randomised controlled trial planned to last for 7 years. Here we present the prespecified interim analysis, done after each participant had completed her month 48 visit.

We enrolled healthy women older than 25 years from Australia, Canada, Mexico, the Netherlands, Peru, Philippines, Portugal, Russia, Singapore, Thailand, the UK, and the USA. Enrolment was stratified by age, with about 45% of participants in each of the 26–35 years and 36–45 years strata, and about 10% in the 46 years and older stratum. Previous HPV exposure was not an exclusion criterion, and each stratum could include a subset of up to 15% of women with a history of HPV-associated infection or disease (defined as two or more abnormal smears in sequence, abnormal colposcopy, or biopsy or treatment of the cervix after abnormal smear or colposcopy findings). The proportion of 15% was chosen to balance inclusion of women likely to have a strong interest in vaccination (and thus to represent a real-world setting) with the need to ensure that most of the women enrolled would be eligible for assessment of per-protocol prophylactic vaccine efficacy. We excluded women who were pregnant or breastfeeding, or who had a chronic or autoimmune disease or immunodeficiency. There was no restriction on the lifetime number of sexual partners.

The trial has been approved by each site's independent ethics committee or institutional review board, and is being done in accordance with the Declaration of Helsinki (1996), the International Conference on Harmonisation Good Clinical Practice guidelines, and the US 21 Code of Federal Regulation. An independent data monitoring Committee is overseeing the study. All participants provided written informed consent.

Randomisation and masking

We randomly assigned women (1:1) to receive either the HPV 16/18 AS04-adjuvanted vaccine (Cervarix, GlaxoSmithKline Biologicals, Rixensart, Belgium)[19] or control (aluminium hydroxide) in a 0–1–6 month schedule. Randomisation was internet based, and used a randomisation blocking scheme (1:1 ratio), with the randomisation list generated by the funder by use of a standard SAS program. The randomisation algorithm used a minimisation process that accounted for region, age stratum, and HPV history. All participants, investigators, and study staff involved in data cleaning or interpretation were masked to treatment allocation and study results. Vaccine and control were supplied in

Panel 1: **Study cohorts**

Total vaccinated cohort

- Received at least one dose of study vaccine
- Data available for efficacy endpoints (ie, baseline PCR or cytology sample and one further sample available)
- Includes subset of women with history of human papillomavirus (HPV) infection or disease (about 15%)
- Case counting began the day after the first vaccination
- Endpoints assessed irrespective of baseline HPV DNA or serostatus, unless otherwise stated

Total vaccinated cohort for efficacy

- Received at least one dose of study vaccine
- Data available for efficacy endpoints (ie, baseline PCR or cytology sample and one further sample available)
- Negative or low-grade cytology at month 0
- No history of HPV disease
- Case counting began the day after the first vaccination
- HPV 16/18-related endpoints assessed in women DNA-negative and seronegative for corresponding HPV type at month 0

According-to-protocol cohort for efficacy

- Met eligibility criteria and complied with protocol
- Received three doses of study vaccine
- Data available for efficacy endpoints (ie, baseline PCR or cytology sample and one further sample available)
- Negative or low-grade cytology at month 0
- No history of HPV disease
- Case counting began the day after the third vaccination
- HPV 16/18-related endpoints assessed in women DNA-negative and seronegative for corresponding HPV type at month 0 and DNA-negative at month 6, unless otherwise stated
- Non-vaccine type-related endpoints assessed in women DNA-negative for corresponding HPV type at months 0 and 6, irrespective of serostatus

identical prefilled syringes. To maintain masking, an external statistician did the interim analysis. Women were informed that they could leave the trial to receive a licensed HPV vaccine, and that they would have the opportunity to be vaccinated after the trial if they had received the control vaccine.

Children's Hospital at Westmead, Sydney, NSW 2145, Australia
rachel.skinner@health.nsw.gov.au

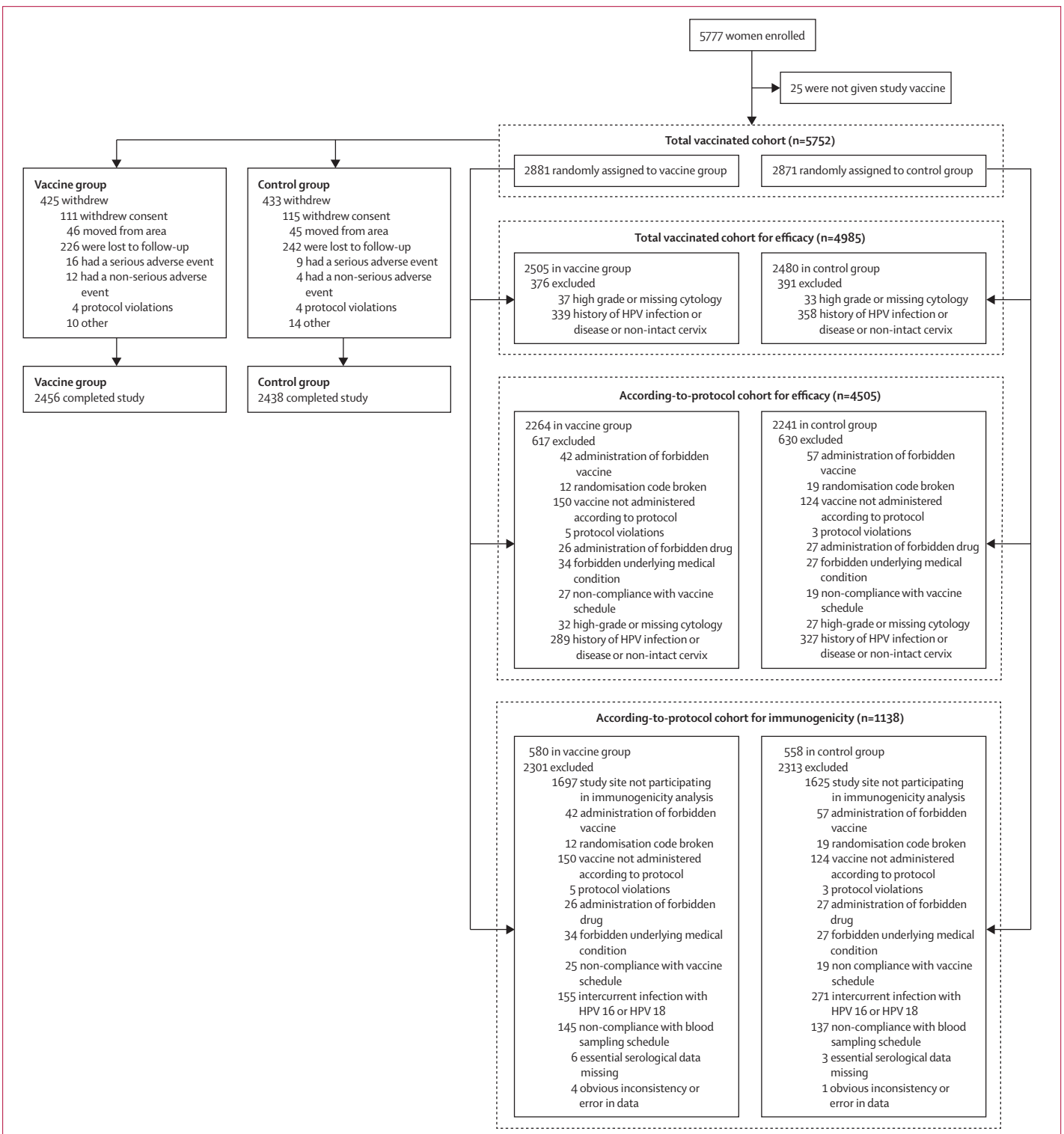


Figure 1: **Study profile**

	According-to-protocol cohort for efficacy		Total vaccinated cohort		Subset with history of HPV infection or disease	
	Vaccine group (n=2264)	Control group (n=2241)	Vaccine group (n=2881)	Control group (n=2871)	Vaccine group (n=345)	Control group (n=360)
Mean age (years)	37·0 (7·2)	37·1 (7·4)	37·0 (7·2)	37·0 (7·3)	36·7 (7·2)	37·0 (7·2)
Age group (years)*						
26–35	996 (44%)	985 (44%)	1275 (44%)	1282 (45%)	153 (44%)	165 (46%)
36–45	1023 (45%)	1004 (45%)	1291 (45%)	1275 (44%)	155 (45%)	157 (44%)
≥46	245 (11%)	252 (11%)	312 (11%)	314 (11%)	36 (10%)	38 (11%)
Region						
Asia-Pacific	742 (33%)	729 (33%)	838 (29%)	830 (29%)	51 (15%)	55 (15%)
Europe	428 (19%)	406 (18%)	569 (20%)	565 (20%)	94 (27%)	100 (28%)
Latin America	584 (26%)	600 (27%)	736 (26%)	738 (26%)	69 (20%)	67 (19%)
North America	510 (23%)	506 (23%)	738 (26%)	738 (26%)	131 (38%)	138 (38%)
Ethnic origin						
White	860 (38%)	853 (38%)	1202 (42%)	1215 (42%)	221 (64%)	238 (66%)
East or southeast Asian	575 (25%)	568 (25%)	629 (22%)	620 (22%)	18 (5%)	15 (4%)
Hispanic	633 (28%)	643 (29%)	805 (28%)	803 (28%)	82 (24%)	77 (21%)
Other	196 (9%)	177 (8%)	245 (9%)	233 (8%)	24 (7%)	30 (8%)
Smoking status						
Smoker	297 (13%)	278 (12%)	399 (14%)	399 (14%)	55 (16%)	65 (18%)
Ex-smoker	281 (12%)	272 (12%)	409 (14%)	383 (13%)	77 (22%)	67 (19%)
Non-smoker	1686 (74%)	1691 (75%)	2070 (72%)	2087 (73%)	213 (62%)	228 (63%)
Missing data	0	0	3 (<1%)	2 (<1%)	0	0
HPV 16 infection status						
DNA-negative and seronegative	1576 (70%)	1555 (69%)	1925 (67%)	1909 (66%)	167 (48%)	186 (52%)
DNA-negative and seropositive	623 (28%)	620 (28%)	849 (29%)	846 (29%)	155 (45%)	145 (40%)
DNA-positive and seronegative	16 (1%)	16 (1%)	32 (1%)	25 (1%)	10 (3%)	4 (1%)
DNA-positive and seropositive	30 (1%)	33 (1%)	49 (2%)	66 (2%)	9 (3%)	20 (6%)
Missing data	19 (1%)	17 (1%)	26 (1%)	25 (1%)	4 (1%)	5 (1%)
HPV 18 infection status						
DNA-negative and seronegative	1625 (72%)	1598 (71%)	2024 (70%)	2001 (70%)	208 (60%)	224 (62%)
DNA-negative and seropositive	587 (26%)	575 (26%)	787 (27%)	785 (27%)	127 (37%)	125 (35%)
DNA-positive and seronegative	7 (<1%)	9 (<1%)	13 (<1%)	14 (<1%)	3 (1%)	3 (1%)
DNA-positive and seropositive	13 (1%)	12 (1%)	19 (1%)	16 (1%)	5 (1%)	2 (1%)
Missing data	32 (1%)	47 (2%)	38 (1%)	55 (2%)	2 (1%)	6 (2%)
HPV 16/18 infection status						
Age 26–35 years						
No evidence of infection†	543 (55%)	560 (57%)	666 (52%)	694 (54%)	54 (35%)	67 (41%)
Evidence of current or past infection‡	433 (43%)	393 (40%)	582 (46%)	547 (43%)	96 (63%)	92 (56%)
Missing data	20 (2%)	32 (3%)	27 (2%)	41 (3%)	3 (2%)	6 (4%)
Age 36–45 years						
No evidence of infection†	583 (57%)	560 (56%)	701 (54%)	682 (53%)	52 (34%)	64 (41%)
Evidence of current or past infection‡	424 (41%)	421 (42%)	570 (44%)	565 (44%)	100 (65%)	89 (57%)
Missing data	16 (2%)	23 (2%)	20 (2%)	28 (2%)	3 (2%)	4 (3%)
Age ≥46 years						
No evidence of infection†	144 (59%)	146 (58%)	180 (58%)	177 (56%)	16 (44%)	18 (47%)
Evidence of current or past infection‡	90 (37%)	102 (40%)	120 (38%)	133 (42%)	20 (56%)	20 (53%)
Missing data	11 (4%)	4 (2%)	12 (4%)	4 (1%)	0	0
History of hormonal contraceptive use						
No	844 (37%)	821 (37%)	1020 (35%)	1002 (35%)	75 (22%)	83 (23%)
Yes	1420 (63%)	1420 (63%)	1861 (65%)	1869 (65%)	270 (78%)	277 (77%)

(Table 1 continues on next page)

	According-to-protocol cohort for efficacy		Total vaccinated cohort		Subset with history of HPV infection or disease	
	Vaccine group (n=2264)	Control group (n=2241)	Vaccine group (n=2881)	Control group (n=2871)	Vaccine group (n=345)	Control group (n=360)
(Continued from previous page)						
History of sexually transmitted infection						
No	1958 (86%)	1978 (88%)	2404 (83%)	2425 (84%)	223 (65%)	234 (65%)
Yes	279 (12%)	251 (11%)	440 (15%)	419 (15%)	113 (33%)	112 (31%)
Uncertain	27 (1%)	12 (1%)	37 (1%)	27 (1%)	9 (3%)	14 (4%)
Number of sexual partners during past year						
0	0	1 (<1%)	2 (<1%)	2 (<1%)	2 (1%)	1 (<1%)
1	1868 (83%)	1824 (81%)	2342 (81%)	2290 (80%)	264 (77%)	264 (73%)
2	116 (5%)	121 (5%)	158 (5%)	179 (6%)	28 (8%)	44 (12%)
≥3	45 (2%)	58 (3%)	70 (2%)	92 (3%)	14 (4%)	20 (6%)
Missing data	235 (10%)	237 (11%)	309 (11%)	308 (11%)	37 (11%)	31 (9%)
Number of lifetime sexual partners						
0	10 (<1%)	19 (1%)	14 (<1%)	21 (1%)	1 (<1%)	0
1	918 (41%)	936 (42%)	1068 (37%)	1093 (38%)	70 (20%)	69 (19%)
2–5	850 (38%)	808 (36%)	1079 (37%)	1041 (36%)	113 (33%)	135 (38%)
6–10	244 (11%)	263 (12%)	366 (13%)	369 (13%)	80 (23%)	64 (18%)
11–15	106 (5%)	93 (4%)	146 (5%)	154 (5%)	29 (8%)	38 (11%)
16–20	51 (2%)	47 (2%)	71 (2%)	70 (2%)	15 (4%)	16 (4%)
>20	85 (4%)	75 (3%)	134 (5%)	121 (4%)	37 (11%)	38 (11%)
Missing data	0	0	3 (<1%)	2 (<1%)	0	0

Data are n (%) or mean (SD). HPV=human papillomavirus. *Three participants in the vaccine group (including one in the subset with a history of HPV infection or disease) were aged 25 years at baseline (included in the total vaccinated cohort and the subset with a history of HPV infection or disease, but excluded from percentage calculations for age group). †DNA-negative and seronegative for both HPV-16 and HPV-18. ‡DNA-positive or seropositive for HPV 16 or HPV 18.

Table 1: **Demographic characteristics and smoking and gynaecological history at baseline**

Procedures

We obtained cytology samples for HPV DNA testing every 6 months and Pap cytology testing every 12 months, as previously described for the PATRICIA trial.[9,10,20] We used a broad spectrum PCR SPF_{10}-DEIA-$LiPA_{25}$ (version 1) assay to test cervical samples and biopsy material for HPV DNA from HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68/73.[21] Additionally, we tested oncogenic HPV-positive samples by multiplex type-specific PCR and reverse hybridisation assay (MPTS12 RHA) to detect HPV types 16, 18, 31, 33, 35, 45, 52, 58, and 59.[22] Treating clinicians followed a standard clinical care algorithm (appendix p 1). The colposcopy method varied across the different countries and was done in accordance with local practices. However, a training and quality assurance programme for study colposcopists was implemented to increase the reliability of colposcopy. All histopathology was done centrally. Biopsy samples were initially reviewed by a routine panel and all samples with a diagnosis of CIN1 or higher were referred to a masked study panel of three expert gynaecological pathologists under the supervision of a fourth pathologist. Agreement on the location and grade of the lesion from at least two panel members was required. An endpoint committee made final case assignments.

Antibody responses against HPV 16 and HPV 18 were determined by ELISA at 6-month intervals up to 24 months and yearly thereafter.[23] Participants recorded solicited symptoms for 7 days and unsolicited symptoms for 30 days after each vaccination. Serious adverse events, new-onset chronic diseases, new-onset autoimmune diseases, medically significant conditions, pregnancy, and pregnancy outcomes were recorded throughout the 48-month follow-up reported here.

Outcomes

The primary endpoint was vaccine efficacy against a combined endpoint of 6-month persistent infection with HPV 16 or HPV 18 (HPV 16/18) or CIN grade 1 or greater (CIN1+) associated with HPV 16/18. The primary endpoint was initially assessed in an analysis that took into account all HPV DNA detected in any component of cervical tissue associated with the lesion. If vaccine efficacy was shown in this analysis, it was further assessed by use of the previously described type-assignment algorithm,[9,20] by which lesions with more than one HPV type were assigned to the HPV type most likely to have caused the lesion.

See Online for appendix

We report the results of selected secondary and exploratory efficacy endpoints of the greatest clinical relevance. These are 6-month and 12-month persistent infection, atypical squamous cells of undetermined significance or greater (ASC-US+), CIN1+, and CIN2+: associated with HPV 16/18; associated with non-vaccine

	Vaccine group*			Control group			Efficacy (97·7% CI)	Number cases prevented per 100 000 woman-years (97·7% CI)
	n	Cases	Rate	n	Cases	Rate		
According-to-protocol cohort for efficacy								
Combined primary endpoint (6-month persistent infection or CIN1+)								
All	1898	7	0·11	1854	36	0·58	81·1% (52·1 to 94·0)	474 (252 to 751)
26–35 years	857	4	0·14	819	23	0·86	83·5% (45·0 to 96·8)	720 (320 to 1247)
36–45 years	827	3	0·11	822	13	0·47	77·2% (2·8 to 96·9)	364 (44 to 779)
≥46 years	214	0	0·00	213	0	0·00	..	0 (–705 to 706)
6-month persistent infection								
All	1859	6	0·09	1822	34	0·55	82·9% (53·8 to 95·1)	459 (245 to 730)
26–35 years	834	3	0·11	800	22	0·83	87·1% (50·4 to 98·2)	721 (341 to 1239)
36–45 years	816	3	0·11	809	12	0·44	75·4% (–7·5 to 96·7)	329 (14 to 734)
≥46 years	209	0	0·00	213	0	0·00	..	0 (–708 to 706)
CIN1+								
All	1898	1	0·02	1854	7	0·11	86·1% (–35·4 to 99·9)	98 (–8 to 248)
CIN2+								
All	1898	0	0·00	1854	4	0·06	100% (–100·7 to 100·0)	65 (–17 to 192)
ASC-US+								
All	1898	2	0·03	1854	31	0·51	93·7% (71·5 to 99·5)	475 (291 to 731)
HSIL	1898	0	0·00	1854	0	0·00	..	0 (–82 to 84)
LSIL	1898	1	0·02	1854	18	0·29	94·6% (59·1 to 99·9)	278 (142 to 484)
Total vaccinated cohort for efficacy								
Combined primary endpoint (6-month persistent infection or CIN1+)								
All	2038	12	0·16	1996	45	0·60	74·0% (45·4 to 88·9)	443 (229 to 697)
26–35 years	923	8	0·23	894	30	0·91	74·4% (36·2 to 91·3)	674 (278 to 1156)
36–45 years	885	4	0·12	877	13	0·39	69·7% (–14·3 to 94·4)	271 (–8 to 618)
≥46 years	229	0	0·00	225	2	0·23	100% (–710·3 to 100·0)	231 (–347 to 1000)
6-month persistent infection								
All	2022	10	0·13	1987	43	0·57	77·4% (49·7 to 91·1)	443 (237 to 690)
26–35 years	911	6	0·17	891	29	0·88	80·1% (45·1 to 94·4)	703 (328 to 1173)
36–45 years	881	4	0·12	872	12	0·36	67·2% (–26·6 to 94·0)	242 (–34 to 580)
≥46 years	229	0	0·00	224	2	0·23	100% (–710·2 to 100·0)	231 (–348 to 1001)
CIN1+								
All	2038	2	0·03	1996	8	0·11	75·5% (–49·4 to 98·3)	80 (–19 to 209)
CIN2+								
All	2038	1	0·01	1996	5	0·07	80·4% (–125·3 to 99·8)	53 (–30 to 165)
ASC-US+								
All	2037	5	0·07	1995	35	0·47	86·1% (59·8 to 96·5)	402 (229 to 625)
HSIL	2037	0	0·00	1995	1	0·01	100% (–8330·2 to 100·0)	13 (–54 to 93)
LSIL	2037	4	0·05	1995	20	0·27	80·4% (33·1 to 96·2)	215 (76 to 394)
Total vaccinated cohort								
Combined primary endpoint (6-month persistent infection and CIN1+)								
All	2772	90	0·89	2779	158	1·59	43·9% (23·9 to 59·0)	698 (346 to 1065)
26–35 years	1225	63	1·46	1243	97	2·26	35·4% (5·8 to 56·1)	800 (140 to 1482)
36–45 years	1245	23	0·50	1229	48	1·07	53·4% (15·7 to 75·2)	569 (156 to 1022)
46+ years	300	4	0·35	307	13	1·14	69·3% (–15·6 to 94·3)	792 (–33 to 1801)
6-month persistent infection								
All	2767	71	0·70	2776	132	1·32	47·0% (25·4 to 62·7)	620 (303 to 952)
26–35 years	1221	48	1·10	1242	78	1·80	38·7% (5·9 to 60·6)	695 (115 to 1301)
36–45 years	1244	19	0·41	1228	43	0·95	57·0% (18·2 to 78·5)	543 (159 to 971)
≥46 years	300	4	0·35	306	11	0·96	63·6% (–44·4 to 93·4)	613 (–186 to 1566)

(Table 2 continues on next page)

oncogenic HPV types individually or in combination; irrespective of HPV infection for ASC-US+, CIN1+, and CIN2+ (includes all samples, irrespective of the HPV type identified, and samples in which no HPV DNA was detected). We did post-hoc assessments of high-grade squamous intraepithelial lesion and low-grade squamous intraepithelial lesion associated with HPV 16/18, and the number of cases prevented. 6-month and 12-month persistent infection, ASC-US+, CIN1+, and CIN2+ were defined as previously described.[9,10,20] We also assessed immunogenicity and safety endpoints.

Statistical analysis

Efficacy analyses were done in the according-to-protocol cohort for efficacy, the total vaccinated cohort, and the total vaccinated cohort for efficacy (panel 1). The according-to-protocol cohort for efficacy was the primary analysis cohort. The total vaccinated cohort included a subset of women (15%) with a previous history of HPV infection or disease. Efficacy analyses of this subset and the total vaccinated cohort excluding this subset are also presented separately; however, the study was not powered to show vaccine efficacy in these post-hoc, exploratory analyses.

The type I error for the entire study period was 5%, distributed as 2·3% for the interim analysis (97·7% CI) and 3·8% for the final analysis (96·2% CI). The target enrolment of 5400 women was estimated to provide about 3200 women assessable for the primary endpoint, assuming 30% of participants were non-assessable and assuming an annual attack rate of 0·7% for HPV 16/18 persistent infection or CIN1+. This attack rate is about a third of that noted in studies of women aged 15–25 years,[9,11] because the attack rate is expected to be much lower in women older than 25 years. On this basis, this interim analysis had 80% power to show 80% vaccine efficacy against the primary endpoint, with the lower limit of the 97·7% CI greater than 30%.

We calculated vaccine efficacy using a conditional exact method. This method computes an exact CI around the rate ratio (ratio of the event rates in the

	Vaccine group*			Control group			Efficacy (97·7% CI)	Number cases prevented per 100 000 woman-years (97·7% CI)
	n	Cases	Rate	n	Cases	Rate		
(Continued from previous page)								
CIN1+								
All	2740	35	0·34	2737	56	0·55	37·8% (–3·2 to 63·1)	209 (–5 to 433)
CIN2+								
All	2740	32	0·31	2737	45	0·44	29·1% (–22·5 to 59·6)	129 (–69 to 335)
ASC-US+								
All	2736	38	0·37	2734	88	0·87	57·2% (32·9 to 73·3)	498 (252 to 764)
HSIL	2736	3	0·03	2734	9	0·09	66·8% (–59·4 to 95·7)	59 (–23 to 158)
LSIL	2736	20	0·20	2734	45	0·44	55·8% (17·2 to 77·4)	247 (70 to 442)
Subset with history of HPV infection or disease								
Combined primary endpoint (6-month persistent infection or CIN1+)								
All	327	17	1·47	352	35	2·94	49·9% (–0·3 to 76·2)	1466 (87 to 2950)
26–35 years	144	12	2·44	162	19	3·72	34·5% (–59·0 to 74·5)	1286 (–1302 to 3994)
36–45 years	147	4	0·75	153	12	2·19	65·7% (–32·3 to 93·7)	1442 (–275 to 3468)
≥46 years	35	1	0·78	37	4	3·00	74·1% (–246·3 to 99·8)	2226 (–2542 to 7839)
6-month persistent infection								
All	326	12	1·03	352	26	2·14	52·0% (–9·3 to 80·5)	1110 (–60 to 2387)
26–35 years	144	9	1·79	162	12	2·27	20·9% (–134·9 to 74·8)	476 (–1714 to 2699)
36–45 years	146	2	0·37	153	11	2·00	81·4% (–1·9 to 98·7)	1628 (161 to 3543)
≥46 years	35	1	0·78	37	3	2·18	64·4% (–516·1 to 99·7)	1409 (–3277 to 6542)
CIN1+								
All	322	7	0·59	347	16	1·29	54·0% (–34·7 to 86·5)	697 (–228 to 1725)
CIN2+								
All	322	7	0·59	347	11	0·88	32·4% (–121·6 to 81·4)	284 (–587 to 1191)
ASC-US+								
All	321	6	0·51	347	19	1·53	66·9% (1·9 to 91·0)	1021 (102 to 2095)
HSIL	321	0	0·00	347	3	0·24	100% (–264·4 to 100·0)	236 (–195 to 805)
LSIL	321	3	0·25	347	12	0·95	73·7% (–15·0 to 96·5)	703 (–17 to 1588)

(Table 2 continues on next page)

	Vaccine group*			Control group			Efficacy (97·7% CI)	Number cases prevented per 100 000 woman-years (97·7% CI)
	n	Cases	Rate	n	Cases	Rate		
(Continued from previous page)								
Total vaccinated cohort excluding subset with history of HPV infection or disease								
Combined primary endpoint (6-month persistent infection or CIN1+)								
All	2445	73	0·82	2427	123	1·41	41·9% (18·2 to 59·1)	590 (234 to 960)
26–35 years	1081	51	1·33	1081	78	2·06	35·4% (1·5 to 58·0)	728 (57 to 1427)
36–45 years	1098	19	0·46	1076	36	0·91	49·0% (0·6 to 74·9)	446 (30 to 904)
≥46 years	265	3	0·30	270	9	0·90	66·9% (–58·5 to 95·8)	600 (–221 to 1606)
6-month persistent infection								
All	2441	59	0·66	2424	106	1·21	45·5% (20·6 to 63·1)	549 (225 to 891)
26–35 years	1077	39	1·01	1080	66	1·73	41·6% (6·3 to 64·2)	720 (119 to 1353)
36–45 years	1098	17	0·41	1075	32	0·81	48·6% (–4·2 to 75·9)	393 (–1 to 827)
≥46 years	265	3	0·30	269	8	0·80	62·8% (–87·1 to 95·3)	500 (–308 to 1470)
CIN1+								
All	2418	28	0·31	2390	40	0·45	30·8% (–24·1 to –62·1)	139 (–73 to 361)
CIN2+								
All	2418	25	0·28	2390	34	0·38	27·3% (–36·2 to –61·9)	104 (–94 to 312)
ASC-US+								
All	2415	32	0·36	2387	69	0·78	54·4% (29·0 to 73·0)	424 (173 to 696)
HSIL	2415	3	0·03	2387	6	0·07	50·6% (–186·1 to 94·1)	34 (–54 to 135)
LSIL	2415	17	0·19	2387	33	0·37	49·1% (–2·7 to 76·0)	182 (3 to 378)

Cases are defined as the number of assessable women who reported at least one event; the rate is the number of cases divided by sum of follow-up period (per 100 woman-years). Women included in the analysis of the according-to-protocol cohort for efficacy were DNA-negative at months 0 and 6 and seronegative at month 0 for at least one of human papillomavirus (HPV) types 16 or 18; had negative or low-grade cytology at month 0; and had no history of HPV disease; follow-up began on the day after the third vaccine dose. Women included in the analysis of the total vaccinated cohort for efficacy were DNA-negative and seronegative at month 0 for at least one of HPV types 16 or 18, had negative or low-grade cytology at month 0, and had no history of HPV disease; follow-up began on the day after the first vaccine dose. Women were included in the analysis of the total vaccinated cohort and of the subset with a history of HPV infection or disease irrespective of their HPV DNA or serostatus at month 0; follow-up began on the day after the first vaccine dose. Cervical intraepithelial neoplasia grade 1 or higher (CIN1+) was defined histologically as CIN1, CIN2, CIN3, adenocarcinoma in situ or invasive carcinoma; CIN2+ excluded CIN1. Atypical squamous cells of undetermined significance or greater (ASC-US+) was defined as ASC-US, low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells or high-grade ASC-US—cannot exclude high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells of undetermined significance, or HSIL. *Three participants in the vaccine group were aged 25 years at baseline; they are included in the total vaccinated cohort and the total vaccinated cohort for efficacy, but are counted only in the overall age group.

***Table 2*: Vaccine efficacy and number of cases prevented for the combined primary endpoint, 6-month persistent infection, CIN1+, CIN2+, and ASC-US+ associated with HPV 16/18**

vaccine *vs* control groups) and takes into account the follow-up time of participants within each group. Vaccine efficacy was defined as 1 minus the rate ratio. Significance was defined for the combined primary endpoint in the according-to-protocol cohort for efficacy when the lower limit of the 97·7% CI around the point estimate was greater than 30%, as required by the US Food and Drug Administration for our phase 3 efficacy study (PApillomaTRIal against Cancer In young Adults, NCT00122681). For all other endpoints and cohorts, significance was defined by the lower limit of the 97·7% CI greater than 0%. Event rates were calculated as the number of cases divided by the total follow-up in years, and were expressed per 100 woman-years. FolloΩp in the according-to-protocol cohort for efficacy started on the day after the third vaccine dose, and in the total vaccinated cohort and the total vaccinated cohort for efficacy on the day after the first dose, and ended at the time of the event or, if no event was reported for a participant, at the time of last data available.

We analysed immunogenicity in the according-to-protocol cohort for immunogenicity, which included all women in the immunogenicity subset (vaccinated women from selected study sites) who met the eligibility criteria, complied with the protocol, and had data available for immunogenicity measures. We calculated seropositivity rates (with exact 95% CIs) and geometric mean titres (with 95% CIs) for HPV 16 and HPV 18. We analysed safety in the total vaccinated cohort, calculating the percentage of participants with an adverse event with exact 95% CIs. We did a post-hoc analysis of the number of deaths in each study group and the time of any deaths in relation to the time of vaccination.

We used SAS version 9.2 for all statistical analyses.

This study is registered with ClinicalTrials.gov, number NCT00294047.

Role of the funding source

The study funder designed the study in collaboration with the investigators, and coordinated collection, analysis, and interpretation of data. Investigators from the VIVIANE Study Group obtained data and cared for the study participants. The authors had access to a summary of all the trial data, to all individual patient data from their own institution, and had final responsibility for the decision to submit for publication.

Results

The first participant was enrolled on Feb 16, 2006, and the last study visit for the present analysis took place on Dec 10, 2010. Mean follow-up time was 44·3 months in the total vaccinated cohort and 40·3 months in the according-to-protocol cohort for efficacy (calculated from the day after the first and third dose, respectively). 5752 women were included in the total vaccinated cohort (n=2881 vaccine, n=2871 control). Participant flow and baseline characteristics are shown in figure 1 and table 1.

In the analysis of all age groups, vaccine efficacy was significant for the combined primary endpoint of 6-month persistent infection or CIN1+ associated with HPV 16/18 (81·1%, 97·7% CI 52·1–94·0) and for 6-month persistent infection with HPV 16/18 (82·9%, 53·8–95·1) in the according-to-protocol cohort for efficacy in women who were seronegative and DNA-negative for the corresponding HPV type at baseline (table 2). Significant vaccine efficacy against these endpoints was also seen in the total vaccinated cohort for efficacy and the total vaccinated cohort (table 2). The type-assignment algorithm produced a similar estimate of vaccine efficacy as the standard analysis (82·1%, 59·4–93·3) in the according-to-protocol cohort for efficacy. In women who were seropositive and DNA-negative for either HPV 16 or HPV 18 (or both) at month 0, vaccine efficacy against the combined endpoint associated with HPV 16/18 was 86·4% (30·1–99·0) in the according-to-protocol cohort for efficacy.

When the analysis was stratified by age, vaccine efficacy was significant in women aged 26–35 and 36–45 years for the combined endpoint associated with HPV 16/18 in the according-to-protocol cohort for efficacy and the total vaccinated cohort, and in those aged 26–35 years in the total vaccinated cohort for efficacy (table 2). Only 17 cases of the combined endpoint were recorded in the 46 years and older age group in the total vaccinated cohort (four in the vaccine group and 13 in the control group; table 2). For 6-month persistent infection with HPV 16/18, significant vaccine efficacy was seen in women aged 26–35 years in the according-to-protocol cohort for efficacy and total vaccinated cohort for efficacy, and in women aged 26–35 and 36–45 years in the total vaccinated cohort (table 2). Again, few cases were reported in the 46 years and older age group.

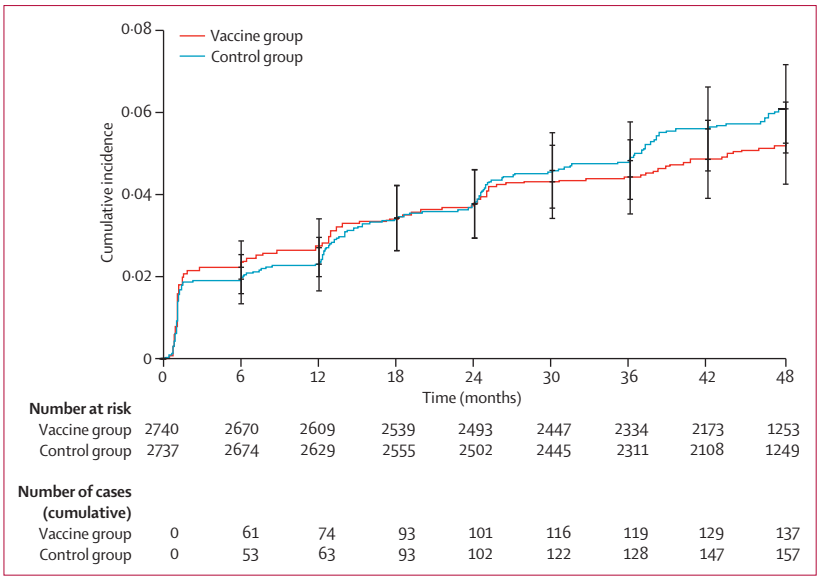
The number of cases of infection or disease prevented per 100 000 woman-years in the 26–35 years age group was similar in all three cohorts for the combined endpoint and for 6-month persistent infection with HPV 16/18; in the 36–45 years age group, more cases were prevented in the total vaccinated cohort than in the according-to-protocol cohort for efficacy or the total vaccinated cohort for efficacy (table 2). Consistently more cases were prevented in the 26–35 years age group than in the 36–45 years age group in all three cohorts, although the difference between the age groups was less substantial in the total vaccinated cohort (table 2). However, the CIs around the point estimates were very wide, limiting any comparative conclusions.

Significant vaccine efficacy was seen against ASC-US+ associated with HPV 16/18 in the according-to-protocol cohort for efficacy, the total vaccinated cohort for efficacy, the total vaccinated cohort, and the subset of women with previous HPV infection or disease (table 2). Few cases of CIN1+ were recorded overall (table 2). In the according-to-protocol cohort for efficacy, vaccine efficacy

	Vaccine group			Control group			Efficacy (97·7% CI)
	n	Cases	Rate	n	Cases	Rate	
HPV 16	2126	6	0·08	2094	34	0·48	82·8% (53·6 to 95·1)
HPV 18	2160	2	0·03	2127	11	0·15	82·2% (2·5 to 98·7)
Non-vaccine A9* species							
HPV 31	2126	4	0·06	2132	19	0·26	79·1% (27·6 to 95·9)
HPV 33	2158	8	0·11	2136	6	0·08	–31·9% (–460·8 to 66·3)
HPV 35	2165	8	0·11	2144	13	0·18	39·3% (–81·2 to 81·5)
HPV 52	2113	33	0·46	2101	38	0·54	14·2% (–51·4 to 51·7)
HPV 58	2152	12	0·16	2135	8	0·11	–48·7% (–393·4 to 51·5)
Composite HPV 31/33/35/52/58	2179	63	0·87	2154	80	1·12	22·9% (–14·4 to 48·4)
Non-vaccine A7* species							
HPV 39	2150	20	0·28	2119	11	0·15	–79·0% (–372·4 to 26·9)
HPV 45	2160	4	0·05	2130	17	0·24	76·9% (18·5 to 95·6)
HPV 59	2158	12	0·16	2126	11	0·15	–7·3% (–208·4 to 62·2)
HPV 68	2138	15	0·21	2128	23	0·32	35·4% (–43·1 to 72·0)
Composite HPV 39/45/59/68	2179	50	0·68	2154	60	0·83	18·0% (–28·9 to 48·1)
Other							
HPV 51	2125	27	0·38	2113	26	0·37	–3·0% (–100·6 to 47·0)
HPV 56	2154	16	0·22	2123	20	0·28	21·4 (–77·4 to 65·9)
HPV 66	2141	27	0·37	2122	27	0·38	0·9% (–91·5 to 48·7)
Composite of 12 non-vaccine oncogenic HPV types	2179	163	2·30	2154	185	2·66	13·7% (–10·8 to 32·8)
Composite of HPV 16, HPV 18, and 12 non-vaccine oncogenic HPV types	2179	170	2·40	2154	217	3·15	23·8% (3·4 to 40·0)

Cases are defined as the number of assessable women who reported at least one event; the rate is the number of cases divided by sum of follow-up period (per 100 woman-years). Women included in the analysis of the according-to-protocol cohort for efficacy were DNA-negative at months 0 and 6 and seronegative at month 0 for the corresponding human papillomavirus (HPV) type; had negative or low-grade cytology at month 0; and had no history of HPV disease; follow-up began on the day after the third vaccine dose. Oncogenic HPV types tested for were HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. *A9 and A7 refer to the classification of HPV species in accordance with the International Committee on Taxonomy of Viruses; A9 species are related to HPV 16 and A7 species are related to HPV 18.

Table 3: **Vaccine efficacy against 6-month persistent infection with vaccine and non-vaccine oncogenic HPV types (according-to-protocol cohort for efficacy)**



Number at risk									
Vaccine group	2740	2670	2609	2539	2493	2447	2334	2173	1253
Control group	2737	2674	2629	2555	2502	2445	2311	2108	1249
Number of cases (cumulative)									
Vaccine group	0	61	74	93	101	116	119	129	137
Control group	0	53	63	93	102	122	128	147	157

Figure 2: **Cumulative incidence of CIN1+ irrespective of HPV DNA status (total vaccinated cohort)**
Women were included in the analysis of the total vaccinated cohort irrespective of their human papillomavirus (HPV) DNA status or serostatus at month 0; follow-up began on the day after the first vaccine dose. Cervical intraepithelial neoplasia grade 1 or higher (CIN1+) was defined histologically as CIN1, CIN2, CIN3, adenocarcinoma in situ, or invasive carcinoma.

against CIN1+ associated with HPV 16/18 was not significant in women who were DNA-negative and seronegative at baseline, but was significant in women who were DNA-negative at baseline irrespective of serostatus (91·1%, 97·7% CI 25·4–99·9). Few cases of CIN2+ were recorded (table 2).

The small number of women (and cases) included in the subset of participants with previous HPV infection or disease precluded assessment of vaccine efficacy. The vaccine group had about half the number of cases of the control group for the HPV 16/18-associated combined primary endpoint, 6-month persistent infection, and CIN1+, and about a third of the number for ASC-US+ (table 2). In an analysis of the total vaccinated cohort excluding the subset of women with previous HPV infection or disease, we noted similar vaccine efficacy to the total vaccinated cohort inclusive of these women (table 2).

Vaccine efficacy was significant for the analysis of 6-month persistent infection with HPV 31 (79·1%, 97·7% CI 27·6–95·9), HPV 45 (76·9%, 18·5–95·6), and a composite of HPV 16, HPV 18, and 12 non-vaccine oncogenic HPV types (23·8%, 3·4–40·0) in the according-to-protocol cohort for efficacy (table 3). Additionally, significant vaccine efficacy was shown against 6-month persistent infection with HPV 45, HPV 56, and the composite of vaccine and 12 non-vaccine types in the total vaccinated cohort (appendix p 2). Fewer cases of 12-month than 6-month persistent infection were recorded, and significant vaccine efficacy was only seen against the composite of vaccine and 12 non-vaccine types in the according-to-protocol cohort for efficacy (appendix p 3).

The cumulative incidence curves for CIN1+ irrespective of HPV DNA status started to separate for the vaccine and control groups at roughly 24 months after first vaccination in the total vaccinated cohort (figure 2), with an overall vaccine efficacy of 14·9% (–10·1 to 34·2).

All initially seronegative women had seroconverted for HPV 16 and HPV 18 at month 7 (1 month after the third dose) in the according-to-protocol cohort for immunogenicity (figure 3). Irrespective of age, all women remained seropositive up to month 48 for HPV 16. All 143 initially seronegative women aged 26–35 years remained seropositive for HPV 18 at month 48, as did 155 (99%) of 156 aged 36–45 years and 38 (97%) of 39 aged 46 years and older. Antibody titre was higher in women who were initially seropositive than in those who were initially seronegative (figure 3).

Solicited injection-site symptoms occurred in more women in the vaccine group than in the control group (table 4). General solicited symptoms during the 7-day post-vaccination period occurred slightly more often in the vaccine group than in the control group. The incidence of unsolicited symptoms, serious adverse events, medically significant conditions, new-onset chronic disease, and new-onset autoimmune disease was similar in both groups, and pregnancy outcomes did not differ between groups (table 4). 17 deaths occurred, 14 (<1%) of 2881 women in the vaccine group and three (<1%) of 2871 in the control group; none of the deaths were believed to be related to vaccination. The independent data monitoring committee did an unblinded review of all deaths; the causes of death were very variable and no cluster of disease type was noted (appendix p 4). The mean time between the last vaccination and death was 682 days (SD 321) in the vaccine group and 496 days (424) in the control group (range 67–1191 days for both groups), suggesting no temporal relation between vaccination and death.

Discussion

Our results show significant vaccine efficacy against the primary combined endpoint of 6-month persistent infection or CIN1+ associated with HPV 16/18 in the overall population and in the 26–35 and 36–45 year age strata (panel 2). We also noted significant cross-protection against HPV 31 and HPV 45.

Licensure studies of HPV vaccines, done in young women, have focused largely on prevention of CIN2+. However, for women older than 25 years, it is unlikely that enough new CIN2+ cases would be accrued with feasible sample sizes to provide a meaningful analysis of vaccine efficacy because of lower rates of HPV infection and higher rates of previous exposure to HPV. Since high concordance in estimates of vaccine efficacy against persistent infection and against CIN2+ has been shown,[10] we chose 6-month persistent infection or CIN1+ associated with HPV 16/18 as the primary endpoint in this study. The value of 6-month persistent infection as a

predictor of CIN2+ has been shown[24,25] and is now generally accepted as a surrogate endpoint in vaccine efficacy trials.[26,27]

At the time of this interim analysis, few cases of CIN1+ were recorded, and it is therefore not surprising that vaccine efficacy against CIN1+ alone was not significant. The same conclusion could also be made for CIN2+. Thus, efficacy against the combined endpoint was largely driven by 6-month persistent infection. More cases of ASC-US+ than CIN1+ were seen, with significant vaccine efficacy seen in all cohorts and in the subset of women with previous HPV infection or disease. As the study progresses, we anticipate that the number of CIN1+ cases will increase.

As expected, we noted lower HPV infection rates in VIVIANE compared with the PATRICIA study,[10] which was done in younger women. The rate of 6-month persistent infection with HPV 16/18 in the total vaccinated cohort was 1·32 per 100 woman-years in the control group of VIVIANE, compared with 4·16 in women aged 15–25 years, 3·23 in those aged 18–25 years, and 2·64 in those aged 21–25 years in PATRICIA.[10] In VIVIANE, a higher infection rate was seen in the 26–35 years age group than in the 36–45 years age group. However, we noted no clear pattern of different estimates of vaccine efficacy between the age groups, and CIs around the point estimates were wide.

As expected, vaccine efficacy was higher in the according-to-protocol cohort for efficacy than in the total vaccinated cohort, because the according-to-protocol cohort for efficacy included only women who were DNA-negative and seronegative for the corresponding HPV type. The finding of higher efficacy in the according-to-protocol cohort for efficacy population is in line with findings from previous studies.[9,10,14,18] Prophylactic HPV vaccines have shown no effect on pre-existing infections or lesions.[9,28] However, significant vaccine efficacy was seen in the total vaccinated cohort, despite inclusion of a subset of women with a history of HPV infection or disease (about 15% of the cohort) and substantial baseline HPV 16/18 seropositivity.

Notably, vaccine efficacy against the combined primary endpoint was similar in women who were seropositive and DNA-negative at baseline compared with those who were both DNA-negative and seronegative. A similar finding has been reported previously,[29,30] suggesting that, in practice, the vaccine might be effective for women who are currently HPV DNA-negative, irrespective of past history of exposure.

In a study of the HPV 6/11/16/18 vaccine in women aged 24–45 years, similar estimates of vaccine efficacy were noted for a comparable endpoint (persistent infection with or without HPV disease) related to HPV 16/18, both overall and stratified by age.[17,18] As in VIVIANE, vaccine efficacy was highest in women who were seronegative and DNA-negative for the relevant HPV type at study entry, with a similar reduction in efficacy in the overall population inclusive of women with prevalent HPV infection.[17,18]

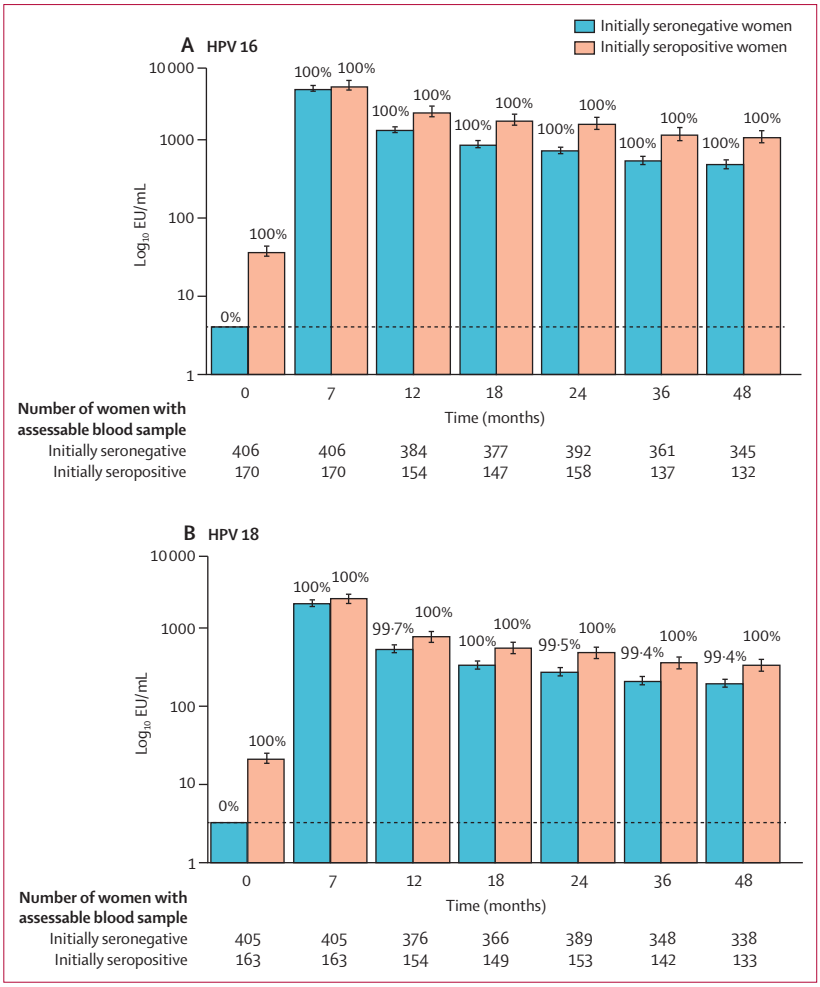


Figure 3: **GMTs and seropositivity rates by HPV type and serostatus at month 0 in women receiving the HPV 16/18 vaccine (according-to-protocol cohort for immunogenicity)**
Bars show log_{10} geometric mean titre (GMT); error bars show 95% CIs. Percentages are the proportion of women seropositive for human papillomavirus (HPV) 16 or HPV 18 antigen. Horizontal lines show the value of half the assay cutoff (8 ELISA units [EU] per mL for HPV 16 and 7 EU/mL for HPV 18), which was assigned to seronegative women for the calculation of the GMT. Seropositivity was defined as antibody titre greater than or equal to the assay threshold. GMTs were calculated by taking the antilog of the mean of the log transformations. Antibody titres below the assay cutoff were given an arbitrary value of half the cutoff.[23] The analysis was stratified by baseline serostatus.

Importantly, we also identified significant cross-protective vaccine efficacy. Our findings are similar to those in the HPV-001 and HPV-007 studies of the HPV 16/18 vaccine,[11] with significant cross-protective vaccine efficacy against HPV 31 and HPV 45. More extensive cross-protection, including against HPV 33 and HPV 51, was seen in the much larger PATRICIA study.[24] Differences in the size of the studies and hence accrual of cases might account for this finding. Cross-protective vaccine efficacy in the study of the HPV 6/11/16/18 vaccine in women of a similar age to participants in the VIVIANE study has not been reported to date.[17,18] Efficacy against HPV 31 and HPV 45 is especially noteworthy, because these types cause roughly 4% and 6% of cervical cancer cases,

	Vaccine group (n=2881)	Placebo group (n=2871)
Safety outcomes		
Solicited injection-site symptoms*† during 7-day post-vaccination period		
All	2443 (85%)	1910 (67%)
Grade 3†	394 (14%)	88 (3%)
General solicited symptoms† during 7-day post-vaccination period		
All	1878 (65%)	1659 (58%)
Related to vaccine	1181 (41%)	1025 (36%)
Grade 3‡	217 (8%)	169 (6%)
Unsolicited symptoms during 30-day post-vaccination period		
All	1154 (40%)	1164 (41%)
Related to vaccine	246 (9%)	192 (7%)
Grade 3§	207 (7%)	184 (6%)
Serious adverse events¶		
All	285 (10%)	267 (9%)
Related to vaccine	5 (<1%)	8 (<1%)
Medically significant conditions||	1169 (41%)	1136 (40%)
New-onset chronic diseases**	142 (5%)	162 (6%)
New-onset autoimmune diseases**	6 (<1%)	7 (<1%)
Deaths††	14 (<1%)	3 (<1%)
Pregnancy outcomes‡‡		
Total pregnancies	357	358
Ongoing pregnancies	2 (1%)	1 (<1%)
Normal infant	257 (72%)	250 (70%)
Congenital anomaly§§	4 (1%)	7 (2%)
Spontaneous abortion¶¶	67 (19%)	67 (19%)
Elective termination¶¶	20 (6%)	23 (6%)
Therapeutic abortion	0	1 (<1%)
Ectopic pregnancy	5 (1%)	6 (2%)
Stillbirth	0	2 (1%)
Lost to follow-up	2 (1%)	1 (<1%)

Data are n or n (%). *All local (injection-site) symptoms were classified as related to vaccination. †Pain (grades: 0=absent; 1=painful on touch; 2=painful when limb is moved; 3=prevents normal activity) or redness or swelling (grades: 0=none; 1=>0 to 20 mm; 2=>20 to 50 mm; 3=>50 mm) at the injection site. ‡Headache, fatigue, gastrointestinal symptoms, arthralgia, myalgia, rash (grades: 0=absent; 1=easily tolerated; 2=interferes with normal activity; 3=prevents normal activity); fever (grades: 0=<37·5°C; 1=37·5 to 38·0°C; 2=>38·0 to 39·0°C; 3=>39·0°C); urticaria (grades: 0=normal; 1=distributed on a single body area; 2=distributed on 2–3 body areas; 3=distributed on at least four body areas). §Grades: 1=easily tolerated; 2=interferes with normal activity; 3=prevents normal activity. ¶Serious adverse events were defined as any untoward medical occurrence that results in death, is life-threatening, requires or prolongs hospital admission, or results in disability or incapacity, or a congenital anomaly or birth defect in the study participant's offspring. ||Medically significant conditions were defined as adverse events prompting either emergency room visits, physician visits that are not routine or related to common diseases, or serious adverse events that are not related to common diseases. **A predefined list of potential new-onset chronic diseases was reviewed by the independent data monitoring committee; on the basis of this prespecified list, the clinical database was searched for all potential new-onset chronic diseases and reviewed by a (blinded) GlaxoSmithKline physician before data analysis; an event was regarded as a potential new-onset chronic disease if it had not been recorded in the previous medical history of the participant (ie, new-onset) or if symptoms were characteristic of a new-onset chronic disease; a separate list, restricted to potential autoimmune events, was also reviewed by the independent data monitoring committee and was used by the GlaxoSmithKline safety physician to identify new-onset autoimmune diseases. ††p=0·0126 (vaccine *vs* control; Fisher's exact test); no deaths were regarded as related to vaccination in either the vaccine group or control group. ‡‡Percentages calculated from total number of pregnancies in each group. §§Congenital anomalies were defined as structural-morphological, chromosomal, or genetic anomalies. ¶¶Of the elective terminations, one fetus with a congenital anomaly was identified in the vaccine group; of the spontaneous abortions, one fetus with a congenital anomaly was identified in the control group.

Table 4: **Safety and pregnancy outcomes up to month 48 (total vaccinated cohort)**

respectively.[2] HPV 45 is particularly associated with adenocarcinoma (12% of cases),[2] the incidence of which is rising in many countries.[31]

Other than injection site symptoms, the number of adverse events reported in the vaccine group was similar to the placebo control, with no increase in medically significant conditions, new-onset chronic disease, or new-onset autoimmune disease. Pregnancy outcomes were also similar in the vaccine and control groups. These findings are similar to those from pooled safety data for more than 30 000 trial participants.[32] Because of an unexpected imbalance in the number of deaths, the independent data monitoring committee assessed the deaths in accordance with causality criteria endorsed by WHO.[33] They concluded that the imbalance was probably caused by chance, since they could identify no clustering in the nature of the cause of death, no consistency with other safety findings from this or any other studies, no temporal relation between vaccination and death, and no medical grounds to support a causal link to the vaccine.

Panel 2: Research in context

Systematic review

We have systematically followed the scientific literature related to human papillomavirus (HPV) vaccination studies before and during the study, and during the development of this report (1997–2013). The amount of relevant scientific literature has increased over the years and we have used our knowledge and expertise to select the publications we believed to be most relevant to the present report. Additionally, we focused particularly on studies of HPV vaccine clinical efficacy, epidemiology, and natural history in women in older age groups than those targeted in most existing population-based HPV vaccination programmes. One previous clinical trial[17,18] has been done to assess an HPV vaccine (the HPV 6/11/16/18 vaccine) in adult women aged 24–45 years with no previous history of HPV infection or disease. The results showed high vaccine efficacy at 48 months of follow-up in the per-protocol cohort and moderate vaccine efficacy in the intention-to-treat cohort, for a combined endpoint of HPV 16/18 infection and disease.

Interpretation

In this interim analysis of the VIVIANE study, we present the first published data for the clinical efficacy of the HPV 16/18 AS04-adjuvanted vaccine against HPV 16/18 infection and related cervical intraepithelial neoplasia of grade 1 or higher in women older than 25 years, at 48 months of follow-up. Importantly, our study included an explicit subset of women with a history of HPV infection or disease. Our results show that the HPV 16/18 vaccine is efficacious against infections and cervical abnormalities associated with the vaccine types, as well as infections with the non-vaccine HPV types 31 and 45. Our findings lend support the contention that women older than 25 years can benefit from HPV vaccination, including those who have been previously exposed to HPV.

The kinetics of the antibody response were broadly similar to those seen in previous studies; HPV 16 and HPV 18 antibody concentrations peaked 1 month after the third vaccination and reached a plateau thereafter.[9,11] Little difference was noted between age groups in immunogenicity of the vaccine. Peak antibody concentrations and the concentrations at month 48 were similar in VIVIANE to those previously reported in women aged 15–25 years.[11] All women remained seropositive for HPV 16 and more than 99% remained seropositive for HPV 18.

An important strength of VIVIANE is that it investigated a broad population of women, including 15% with a history of HPV infection or disease, with no restriction on lifetime number of sexual partners. In a population older than 25 years in Victoria, Australia, the prevalence of high-grade lesions was about 1% and of low-grade lesions about 5%.[34] In a US population, the cumulative incidence of an abnormal Pap smear over 5 years of follow-up for women older than 29 years was roughly 4% in women who were negative for oncogenic HPV at baseline, compared with 15% in those who were positive for oncogenic HPV.[35] Therefore, we believe that the proportion of women in the total vaccinated cohort with a history of HPV infection or disease (15%) in our study was high compared with a general population of women older than 25 years, and that inclusion of this proportion was a conservative approach, ensuring that this group was appropriately represented in our sample. Indeed, the baseline exposure to HPV 16/18 in our total vaccinated cohort was higher than would be expected in a general population of this age,[3] and higher than that of the study population assessed in the other published vaccine efficacy trial in this age group.[17] This higher baseline exposure to HPV, the lower infection rate compared with younger women, and the small sample size compared with PATRICIA limited the analysis and choice of endpoints for this interim analysis. Although we did a separate analysis in the subset of women with evidence of previous HPV infection or disease, the study was not powered to assess vaccine efficacy in this group specifically. We anticipate that vaccine efficacy against a greater range of endpoints will be shown at the end of study follow-up.

Adolescent girls before sexual debut will probably continue to be the main priority for population-level, publicly funded vaccination programmes. Because of the high incidence of HPV infection in adolescence and young adulthood, with a reduction thereafter, modelling has shown substantially lower cost-effectiveness of vaccination programmes that include adult women compared with programmes that target adolescent girls and young adult women.[36,37] However, cost-effectiveness models need to make assumptions about features of the natural history of HPV-related cervical disease that are not well established, such as duration of natural immunity, which affect the cost-effectiveness of vaccination in adult women.[38] New infections occur during adult life, albeit at lower rates than at younger ages, and evidence exists to show that women can also experience reinfection or reactivation of latent infection due to the same oncogenic HPV type.[39] Indeed, women with detectable natural antibodies are not reliably protected against reinfection,[40] and HPV vaccination of women seropositive to HPV 16, HPV 18, or both has been shown to prevent reinfection and disease in young women.[29,30] These studies provide support for the contention that vaccination of women beyond young adulthood will provide benefit in a real-world setting.[41]

In conclusion, the results from this interim analysis of the VIVIANE trial show the efficacy of the HPV 16/18 vaccine against infection and cytological abnormalities associated with vaccine types and infection with the non-vaccine types, HPV 31 and HPV 45. In our interim analysis of VIVIANE, women aged 25–45 years who were HPV DNA-negative derived protective benefit. Because of the small number of cases in women older than 45 years, we could not conclude vaccine efficacy in this age group. Understanding the potential benefit for individual women older than 25 years will aid clinicians in making informed recommendations.

Contributors

AS, BG, BR, EL-P, FS, M-PD, SMG, SRS, and WQ were the core writing team for the report. All authors contributed to study design, acquisition of data or statistical analyses, and interpretation of data. All authors reviewed and commented on a draft version of the report and gave final approval for it to be submitted for publication.

VIVIANE Study Group collaborators

Principal investigators and coprincipal investigators—Australia: S M Garland, S R Skinner, and T Stoney. Canada: C Bouchard, L Ferguson, M Ferguson, S Frank, G Girard, S McNeil, D M Money, B Romanowski. Mexico: A Cruz-Valdez, E Lazcano-Ponce, and J Salmerón. Netherlands: B T Harmsel, G G Kenter, and R Verheijen. Peru: H Gómez Moreno. Philippines: J E Raymundo, M R Del Rosario-Raymundo, and S Villanueva. Portugal: E Bartolo, V Patricio, D Pereira da Silva, and I Santos. Russia: I Gogotadze, G Minkina, V Prilepskaya, V Romanenko, and A Savicheva. Singapore: K L Fong, A Ilancheran, T Y K Lim, S C Quek, and E H Tay. Thailand: S Angsuwathana. UK: M Cruickshank, H Kitchener, N Savani, A Szarewski, and A Tristram. USA: B Allen, N Chakhtoura, C Chambers, A Chatterjee, L R DeMars, R P Edwards, P Fine, B Fox, C Hansen, D M Harper, J Hedrick, M Levin, M G Martens, J Rosen, M Sperling, J Stapleton, C S Thoming, W Utian, and C Wheeler.

GlaxoSmithKline clinical study support—B Colau, S Poncelet, G Edwards, M Lascar, A-S Perreaux, and E Kolp.

Laboratory contributors—E Alt, B Iskaros, A Limaye, R D Luff, and M McNeeley (Quest Diagnostics Clinical Trials, Teterboro, NJ, USA); and A Molijn, W Quint, L Struijk, M Van de Sandt, and L J Van Doorn (DDL Diagnostic Laboratory, Rijswijk, Netherlands).

Endpoint committee—N Kiviat, K P Klugman, and P Nieminen.

Independent data monitoring committee—C Bergeron, E Eisenstein, R Karron, R Marks, T Nolan, and S K Tay.

Declaration of interests

DD, GD, FS, KH, BG, and M-PD are employees, and DM a former employee, of the GlaxoSmithKline group of companies. DD, GD, KH, BG and FS own stock in GlaxoSmithKline Biologicals SA; M-PD owns shared options in GlaxoSmithKline Biologicals SA; and GD holds a relevant patent with GlaxoSmithKline Biologicals SA. MJL holds a patent with Merck Sharp & Dohme. All investigators at clinical study sites were funded by GlaxoSmithKline Biologicals SA through their institutions to do the study. MM has received research funding from GlaxoSmithKline

Biologicals SA through his institution. EL-P received funding from GlaxoSmithKline Biologicals SA and Merck Sharp & Dohme to participate as principal investigator in human papillomavirus (HPV) efficacy trials. AC and MJL received grants or funding for research from GlaxoSmithKline Biologicals SA and Merck Sharp & Dohme, and AC from MedImmune, Sanofi Pasteur MSD, Novartis, and Wyeth. MJL chaired a GlaxoSmithKline Biologicals SA adjudication committee, has received consulting fees from MedImmune and Novartis, and receives funding from GlaxoSmithKline Biologicals SA to do HPV vaccine trials. AC has received payment for service on speakers' bureaus and advisory boards from GlaxoSmithKline Biologicals SA, MedImmune, Merck Sharp & Dohme, Sanofi Pasteur MSD, and Novartis. MRDR-R has received research funding and support for travel from GlaxoSmithKline Biologicals SA. AS has received consulting fees, payment for lectures, and travel reimbursements from GlaxoSmithKline Biologicals SA and Merck Sharp & Dohme; she also received, through her institution, research funding from GlaxoSmithKline Biologicals SA. RHMV received money via her institution for participating in the study. BR received research grants from GlaxoSmithKline Biologicals SA, and, through her professional corporation (Dr Barbara Romanowski Professional Corporation, Edmonton, AB, Canada), travel support to attend scientific meetings and honoraria for speaking engagements and participation in advisory board meetings. HK was funded by GlaxoSmithKline Biologicals SA (through his institution) to participate in the study. CB has received travel grants to attend international scientific meetings and honoraria from GlaxoSmithKline Biologicals SA and fees for lectures to family physicians from Merck Sharp & Dohme; through her institution she was funded by Merck Sharp & Dohme to participate in a study related to an HPV vaccine. She has also received payment for service on speakers' bureaus and advisory boards from GlaxoSmithKline Biologicals SA and Merck Sharp & Dohme. Through her institution, SRS received funding from GlaxoSmithKline Biologicals SA and bioCSL to do investigator-driven research projects; her institution has received reimbursement from GlaxoSmithKline Biologicals SA for travel expenses (to present data from clinical trials of HPV vaccines at scientific meetings) and she has received honoraria from GlaxoSmithKline Biologicals SA for participation in global advisory boards. WQ received grants related to a research collaboration with GlaxoSmithKline Biologicals SA, via a contract research organisation (DDL Diagnostic Laboratory, Voorburg, Netherlands). Through his institution, JS has received research funding from GlaxoSmithKline Biologicals SA, Qiagen, Merck Sharp & Dohme, and Sanofi Pasteur MSD. SMG has received advisory board fees and grant support from CSL and GlaxoSmithKline Biologicals SA; funding through her institution to do HPV vaccine studies for GlaxoSmithKline Biologicals SA, Merck Sharp & Dohme, and Sanofi Pasteur MSD; and lecture fees and from Merck Sharp & Dohme and Sanofi Pasteur MSD. She is a member of the Merck global advisory board and the Merck scientific advisory committee for HPV. SCQ has received payment for service on on speakers' bureaus and advisory boards from GlaxoSmithKline Biologicals SA and Merck Sharp & Dohme, as well as travel support from GlaxoSmithKline Biologicals SA. DPdS, DMM, AI, JTS, KLF, and MEC declare no competing interests.

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