

Prior human papillomavirus-16/18 AS04-adjuvanted vaccination prevents recurrent high grade cervical intraepithelial neoplasia after definitive surgical therapy: *Post-hoc* analysis from a randomized controlled trial

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Abbreviations: AGC: atypical glandular cells; ASC-H: atypical squamous cells, cannot exclude HSIL; ASC-US: atypical squamous cells of undetermined significance; CI: confidence interval; ; CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus; ; HSIL: high grade squamous intraepithelial lesion(s); LEEP: : loop electrosurgical excision procedure; LSIL: low grade squamous intraepithelial lesion(s); PATRICIA: PApilloma TRIal against Cancer In young Adults; TVC: total vaccinated cohort; VIN: vulvar intraepithelial neoplasia; VaIN: vaginal intraepithelial neoplasia

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We evaluated the efficacy of the human papillomavirus (HPV)–16/18 AS04-adjuvanted vaccine in preventing HPV-related disease after surgery for cervical lesions in a *post-hoc* analysis of the PApilloma TRIal against Cancer In young Adults (PATRICIA; NCT00122681). Healthy women aged 15–25 years were randomized (1:1) to receive vaccine or control at months 0, 1 and 6 and followed for 4 years. Women were enrolled regardless of their baseline HPV DNA status, HPV-16/18 serostatus, or cytology, but excluded if they had previous or planned colposcopy. The primary and secondary endpoints of PATRICIA have been reported previously; the present *post-hoc* analysis evaluated efficacy in a subset of women who underwent an excisional procedure for cervical lesions after vaccination. The main outcome was the incidence of subsequent HPV-related cervical intraepithelial neoplasia grade 2 or greater (CIN2+) 60 days or more post-surgery. Other outcomes included the incidence of HPV-related CIN1+, and vulvar or vaginal intraepithelial neoplasia (VIN/VaIN) 60 days or more post-surgery. Of the total vaccinated cohort of 18,644 women (vaccine = 9,319; control = 9,325), 454 (vaccine = 190, control = 264) underwent an excisional procedure during the trial. Efficacy 60 days or more post-surgery for a first lesion, irrespective of HPV DNA results, was 88.2% (95% CI: 14.8, 99.7) against CIN2+ and 42.6% (–21.1, 74.1) against CIN1+. No VIN was reported and one woman in each group had VaIN2+ 60 days or more post-surgery. Women who undergo surgical therapy for cervical lesions after vaccination with the HPV-16/18 vaccine may continue to benefit from vaccination, with a reduced risk of developing subsequent CIN2+.

What's new?

Persistent infection with oncogenic human papillomavirus (HPV) is a pre-requisite for cervical cancer, with women who have already undergone treatment for related cervical lesions representing a high-risk group for the subsequent development of cervical cancer. To date, HPV vaccination is not thought to alter the course of disease in women with prevalent type-specific infections or pre-existing lesions at the time of vaccination. This *post-hoc* analysis of a randomized controlled trial however shows that women who undergo surgery for cervical lesions after receiving the HPV-16/18 AS04-adjuvanted vaccine may continue to benefit from vaccination, with a reduced risk of developing subsequent high-grade cervical disease.

Persistent infection with oncogenic human papillomavirus (HPV) is a pre-requisite for cervical cancer,¹ with HPV-16 and 18 accounting for ~70% of cases worldwide.^{2,3} Two vaccines against HPV-16 and –18 have been licensed and are being used in public health vaccination programs. In clinical studies conducted in a broad population of women, including those who were sexually active, these vaccines were highly effective in reducing the incidence of persistent infection and high-grade cervical lesions associated with HPV-16/18 and some non-vaccine oncogenic types and reducing cytological abnormalities and subsequent cervical procedures.^{4–11} However, HPV vaccination is not thought to alter the course of disease in women who have prevalent infection or pre-existing lesions at the time of vaccination.¹²

Women who have previously been treated, or are undergoing treatment, for cervical lesions represent a high-risk group for the subsequent development of cervical cancer (2 to 6-fold higher risk compared to women with normal cytology).^{13–18} This could be because treated women are a high risk group for new HPV infections, or due to residual dysplasia following incomplete ablative therapy.¹⁸ Moreover ablative treatment of a lesion will not necessarily eradicate nearby infection, nor latent infection, which can reactivate and manifest as a productive infection later. Therefore, gynaecologists are interested in whether previously unvaccinated women undergoing treatment for cervical disease can benefit from HPV vaccination.

Phase III studies evaluating HPV vaccine efficacy have routinely excluded women with a prior history of colposcopy, making it impossible to prospectively evaluate efficacy in those already having undergone treatment for cervical disease prior to enrolment. As a surrogate, we conducted a *post-hoc* analysis of the end-of-study data from the PApilloma TRIal against Cancer In young Adults (PATRICIA),^{4,5} to evaluate whether the HPV-16/18 AS04-adjuvanted vaccine reduced the incidence of subsequent cervical lesions, compared with control (hepatitis A vaccine), among those women who underwent an excisional procedure for a first histopathologically confirmed lesion after vaccination.

Methods

Detailed methods for PATRICIA, a Phase III, randomized, double-blind, controlled, efficacy trial, have been reported previously.^{4–7} The trial is registered with clinicaltrials.gov, identifier NCT00122681. The protocol and other materials were approved by independent ethics committees or institutional review boards.

Participants

Healthy women aged 15–25 years at first vaccination, from 135 centers in 14 countries in Asia Pacific, Europe, Latin America and North America, who reported no more than six lifetime sexual partners before study enrolment were

included, regardless of their HPV DNA status, HPV serostatus or cytology at baseline.^{4,5} Women were excluded if they had a history of colposcopy or colposcopy was planned to evaluate abnormal cervical cytology. Written informed consent/assent was obtained from all participants and/or their parents.

Randomization and masking

Participants were randomly assigned (1:1) to receive the HPV-16/18 AS04-adjuvanted vaccine (*Cervarix*®, GSK Vaccines) or a control hepatitis A vaccine (GSK Vaccines) at 0, 1 and 6 months. Both groups were unmasked after the month 48 visit and offered crossover vaccination.

Procedures

Cervical liquid-based cytology samples were collected six-monthly. Samples were tested for HPV DNA using broad-spectrum PCR SPF₁₀-LiPa₂₅ (version 1 based on licensed Innogenetics SPF₁₀ technology; Labo Biomedical Products, Rijswijk, The Netherlands), which tested for 14 oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), and type-specific PCR for HPV-16 and -18.^{4,19}

Cytological examination was performed 12-monthly using the Bethesda 2001 classification system. Colposcopic referral and/or repeat cytology were performed according to a prespecified clinical management algorithm.⁶ All lesions were biopsied and treatment was by excision. Lesion margins were evaluated and if compromised, women were managed according to local medical practice, after which all patients were followed according to the prespecified clinical management algorithm. Visual inspection of the vagina and vulva during gynaecological or colposcopic evaluation was added *via* protocol amendment, after which suspected vulvar intraepithelial neoplasia (VIN) or vaginal intraepithelial neoplasia (VaIN) could result in biopsy, with further management according to local practice. Lesional tissue for all cases of CIN1+, VIN1+ or VaIN1+ was tested for HPV DNA by PCR. Women continued study procedures following cervical therapy with further management according to local practice. Exit colposcopy was performed for all women who had cytologically evident abnormalities (atypical squamous cells of undetermined significance/oncogenic HPV positive by HCII or low-grade squamous intraepithelial lesion) in the 12 months preceding, and including, the month 48 visit.

Biopsy and excisional treatment specimens were fixed in buffered formalin, paraffin embedded, cut, then following placement onto slides, haematoxylin and eosin stained for microscopy. Slides were first examined by a routine panel of histopathologists at Quest Diagnostics (Teterboro, NJ, USA), who provided the diagnosis used for clinical management. Thereafter, slides with a diagnosis of CIN1+, VIN1+ or VaIN1+ were sent to a second panel of three gynaecological histopathologists, masked to vaccine allocation, for endpoint determination using a majority rule.⁴

Statistical analysis

The end-of-study analysis was conducted once all subjects had completed the month 48 visit. The main endpoint for the current *post-hoc* analysis was vaccine efficacy against CIN2+ 60 days or more post-surgery, irrespective of HPV DNA type, in women who underwent an excisional procedure [loop electrosurgical excision procedure (LEEP) or cone] for a first cervical lesion. Other endpoints were vaccine efficacy 60 days or more post-surgery against CIN2+ associated with HPV-16 and/or HPV-18 and against CIN1+, VIN1/VaIN1+, cytologically predicted low grade squamous intraepithelial lesions (LSIL) and high grade squamous intraepithelial lesions (HSIL), irrespective of HPV DNA type or associated with HPV-16 and/or HPV-18.

We selected CIN2+ as the main endpoint for the current *post-hoc* analysis, since vaccine efficacy against CIN2+ was the prospectively defined primary endpoint for this trial.^{4,5} Whilst CIN2+ is widely accepted as a surrogate marker for cervical cancer vaccine efficacy in studies of prophylactic HPV vaccines,²⁰ CIN3 is a more sensitive predictor as the true precursor lesion to cervical cancer. Although CIN2 is classified as an HSIL according to the Bethesda System, it is a poor predictor of progression.²¹ Accordingly, the majority of international guidelines protect adolescents below the age of 20 years from treatment of CIN2, because the clinical course of cervical lesions is different in this age group compared with women in the over 25 years' age group, in addition to the adverse pregnancy outcomes for this younger age group post ablative treatment. This is underscored by our findings that only 3 out of 6 cone specimens contained CIN2+.

The 60-day window was selected with the aim of capturing new rather than residual disease, and for consistency with a similar analysis conducted for the quadrivalent HPV vaccine.²² Sensitivity analyses in which case counting started 30, 90 or 120 days post-surgery were performed. For completeness, as these data have not been presented previously, we also report vaccine efficacy against VIN/VaIN1+ and VIN/VaIN2+ associated with HPV-16 and/or HPV-18 for all women (i.e., not only those who underwent surgical therapy).

Event rates were calculated as the number of cases divided by total follow-up in years for each group and were expressed per 100 person-years. Vaccine efficacy and 95% confidence intervals (CIs) were calculated using a conditional exact method. Results were considered to support statistically significant vaccine efficacy if estimates and 95% CIs were above zero. Follow-up started the day after the first treatment for cervical lesions and ended at the time the outcome occurred, or at the time of the last sample (up to month 48). For VIN/VaIN endpoints for all women, follow-up started the day after first vaccination and ended at the time the outcome occurred. The numbers of subjects with VIN/VaIN1+ and VIN/VaIN2+ 60 days or more post-surgery were summarized, but statistical analyses were not done due to the small number of cases.

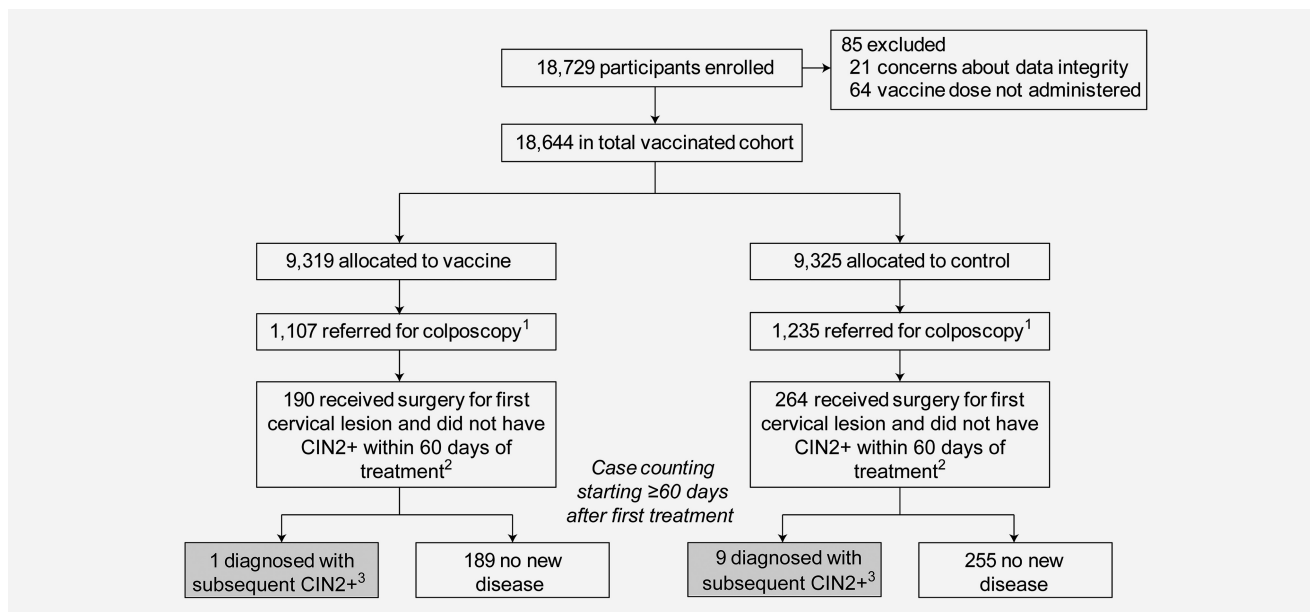


Figure 1. Participant disposition. ¹Number of subjects with at least one colposcopy referral during the study (total number of colposcopy procedures: $n = 2,458$ for vaccine; $n = 2,723$ for control). ²LEEP, cone, or knife. ³CIN2+ at least 60 days after first therapy. Abbreviations: CIN2+: cervical intraepithelial neoplasia grade 2 or greater; LEEP: loop electrosurgical excision procedure.

Endpoints were evaluated in the total vaccinated cohort (TVC), which included all women who received at least one dose of vaccine or control and were evaluable for efficacy (i.e., had a baseline PCR or cytology sample and one further sample available).

Statistical analyses were done with SAS version 9.1 and Proc StatXact-7.

Results

Study population

The TVC of 18,644 women (Fig. 1) was a diverse population (Table 1) including women with evidence of current or previous HPV infection, or with abnormal low-grade or high-grade cytology (Table 2), as reported previously.⁵

At the end-of-study analysis (median follow-up 47.4 months after first vaccine dose), 190 women in the vaccine group and 264 women in the control group had undergone surgical therapy for a first cervical lesion and did not have CIN2+, irrespective of HPV type, within 60 days of treatment (Fig. 1). This group of women was used as the denominator for the primary analysis. The median time from vaccination to first treatment for these women was 19.1 months (range 1.5 to 46.5) and 26.5 months (range 0.8 to 48.3) in vaccine and control groups, respectively. Ten women (vaccine: 1; control: 9) developed CIN2+ 60 days or more post-surgery (Fig. 1).

The demographic characteristics of women who had treatment during the study, and of women who went on to develop CIN2+ post-surgery, were generally similar to the TVC, except a higher proportion reported more than one sexual partner in the year prior to first vaccination (22% for the

TVC, 45% for those receiving treatment, and 60% for women who subsequently developed CIN2+) and had smoked for at least six months (30, 51 and 70%, respectively; Table 1). The proportion of women categorized as non-HPV-naïve at baseline (ie, were DNA positive for at least one of 14 oncogenic HPV types investigated and/or were seropositive for HPV-16 or HPV-18 and/or had abnormal cytology results) was 38% in the TVC, 72% in the cohort that received treatment, and 80% in the cohort that subsequently developed CIN2+ (Table 2). Of the 10 women who subsequently developed CIN2+, 7 were DNA positive for at least one high-risk HPV type and 6 had abnormal cytology at baseline.

Efficacy

Vaccine efficacy against CIN2+ 60 days or more post-surgery was 88.2% (95% CI: 14.8 to 99.7) irrespective of HPV DNA type in the lesion (Table 3). The number of cases of CIN2+ prevented was 1.8 per 100 person-years. There were few cases of HPV-16 and/or HPV-18 CIN2+ and vaccine efficacy for this endpoint was not significant (100% [−63.1 to 100]; Table 3).

The one CIN2+ case in the vaccine group (Case 1; Fig. 2; Table 4) occurred in a 16-year-old woman who, at baseline, was HPV-16 DNA positive and HPV-16 seropositive, with LSIL predicted by cytology. At six months she had HSIL predicted by cytology and was referred for colposcopy. CIN2 (HPV-68 DNA positive) was diagnosed on punch biopsy. She underwent cone biopsy at 8 months and CIN1 was diagnosed; the margins of the excisional material were disease-free. At 14 months she was referred for colposcopy again and VaIN1 (HPV-39/68 positive)

Table 1. Demographic characteristics at baseline

	TVC ¹ (N = 18,644)	Treatment for first lesion during study ² (N = 454)	Subsequent CIN2+ ≥60 days after treatment ³ (N = 10)
Age in years, mean (SD)	20.0 (3.1)	21.1 (4.1)	18.7 (3.3)
Race, n (%)			
Black	693 (3.7)	19 (4.2)	1 (10.0)
East and South East Asian	4,346 (23.3)	13 (2.9)	2 (20.0)
Chinese	1,514 (8.1)	1 (0.2)	0 (0.0)
Hispanic	1,330 (7.1)	26 (5.7)	1 (10.0)
White/Caucasian	10,218 (54.8)	334 (73.6)	6 (60.0)
Other	543 (2.9)	61 (13.4)	0 (0.0)
Ever had sexual intercourse, n (%)			
Yes	15,860 (87.1)	432 (95.2)	10 (100)
No	2,359 (12.9)	22 (4.8)	0 (0.0)
Missing	425	0	0
Number of sexual partners in past year, n (%)			
0	586 (3.7)	7 (1.6)	0 (0.0)
1	11,731 (74.1)	228 (52.9)	4 (40.0)
2	2,275 (14.3)	114 (26.5)	2 (20.0)
≥3	1,231 (7.8)	82 (19.0)	4 (40.0)
Missing	2821	23	0
Smoking status, n (%)			
Never smoked or smoked ≤6 months	12,789 (70.2)	222 (48.9)	3 (30.0)
Smoker for ≥6 months (current or past)	5,432 (29.8)	232 (51.1)	7 (70.0)
Missing	423	0	0

Where data are missing, percentages are calculated out of available data.

¹Nine thousand three hundred nineteen women in vaccine group and 9,325 women in control group.

²One hundred and ninety women in vaccine group and 264 women in control group had treatment for a first lesion during the study without occurrence of CIN2+ within 60 days of first treatment.

³One woman in vaccine group and 9 women in control group had CIN2+ 60 days or more after first treatment.

Abbreviations: CIN2+: cervical intraepithelial neoplasia grade 2 or greater; n (%): number (percentage) of subjects in given category; SD: standard deviation; TVC: total vaccinated cohort.

was diagnosed on punch biopsy. Punch biopsy was repeated at 19 months and CIN2 (HPV-39 positive) and VaIN3 (HPV-68 positive) were diagnosed. She underwent LEEP and was found to have squamous metaplasia (no high risk HPV type detected). No further treatment was done and no abnormality was detected on exit colposcopy at month 60.

In five of the 10 women who developed CIN2+ post-surgery, the HPV genotype found in the new cervical lesion was the same as one of the types found in the first lesion (Cases 4, 6, 7, 8 and 10: Fig. 2; Table 4). The histological margins of excisional material for the first lesion were disease-free for six women (Cases 1, 3, 5, 6, 8 and 9) and compromised for the remaining four (Cases 2, 4, 7 and 10). Two of the women with disease-free histological margins had a new lesion which contained at least one of the HPV genotypes found in the first lesion (Cases 6 and 8). Three of four women with compromised margins (Cases 4, 7 and 10)

had at least one of the HPV genotypes found in the first lesion.

Vaccine efficacy was demonstrated against CIN1+ associated with HPV-16 and/or HPV-18 after surgical therapy (100% [26.1 to 100]), but not against CIN1+ irrespective of HPV genotype in the lesion (42.6% [−21.1 to 74.1]; Table 3). Significant vaccine efficacy was also shown against LSIL associated with HPV-16 and/or HPV-18 (89.5% [21.6 to 99.8], but not against LSIL irrespective of HPV genotype (−30.5% [−142.7 to 29.0]; Table 3). There were only a small number of cases of HSIL (four cases irrespective of HPV DNA and one case associated with HPV-16 and/or HPV-18, all in the control group) and significant vaccine efficacy was not attained (Table 3).

Vaccine efficacy against external genital lesions associated with HPV-16 and/or HPV-18 for all women in the TVC, regardless of whether they underwent surgical therapy, was 73.1% (36.3 to 90.1) for VIN/VaIN1+ (seven and 26 cases in

Table 2. HPV infection and disease status at baseline

	TVC ¹ (N = 18,644) n (%)	Treatment for first lesion during study ² (N = 454) n (%)	Subsequent CIN2+ ≥60 days after treatment ³ (N = 10) n (%)
HPV-naïve ⁴			
Yes	11,644 (62.4)	126 (27.8)	2 (20.0)
No	7,000 (37.5)	328 (72.2)	8 (80.0)
Serostatus at baseline			
HPV-16 seropositive	3,099 (16.6)	153 (33.7)	2 (20.0)
HPV-18 seropositive	2,149 (11.5)	66 (14.5)	2 (20.0)
Serostatus and DNA status at baseline			
HPV-16 seropositive and DNA positive	536 (2.9)	91 (20.0)	1 (10.0)
HPV-18 seropositive and DNA positive	190 (1.0)	13 (2.9)	0
Number of DNA positive results ⁵ at baseline for high risk HPV types			
0 positive results	14,861 (79.7)	168 (37.0)	3 (30.0)
1 positive result	2,472 (13.3)	140 (30.8)	3 (30.0)
2 positive results	865 (4.6)	86 (18.9)	3 (30.0)
≥3 positive results	416 (2.2)	60 (13.2)	1 (10.0)
Missing	30 (0.2)	0	0
DNA positive ⁵ at baseline for individual high risk HPV type			
HPV-16	1,004 (5.4)	142 (31.3)	3 (30.0)
HPV-18	433 (2.3)	32 (7.0)	0
HPV-31	417 (2.2)	48 (10.6)	1 (10.0)
HPV-33	182 (1.0)	31 (6.8)	0
HPV-35	133 (0.7)	10 (2.2)	0
HPV-39	379 (2.0)	33 (7.3)	0
HPV-45	161 (0.9)	17 (3.7)	0
HPV-51	764 (4.1)	54 (11.9)	3 (30.0)
HPV-52	653 (3.5)	53 (11.7)	3 (30.0)
HPV-56	317 (1.7)	15 (3.3)	0
HPV-58	225 (1.2)	29 (6.4)	0
HPV-59	185 (1.0)	4 (0.9)	0
HPV-66	434 (2.3)	31 (6.8)	2 (20.0)
HPV-68	326 (1.8)	22 (4.8)	0
Disease status at baseline			
No disease	16,871 (90.5)	268 (59.0)	4 (40.0)
ASC-US	844 (4.5)	63 (13.9)	1 (10.0)
ASC-H	22 (0.1)	13 (2.9)	1 (10.0)
LSIL	846 (4.5)	86 (18.9)	3 (30.0)
HSIL	58 (0.3)	23 (5.1)	1 (10.0)
AGC	9 (0.0)	1 (0.2)	0

¹Nine thousand three hundred and nineteen women in vaccine group and 9,325 women in control group.

²One hundred ninety women in vaccine group and 264 women in control group had treatment for a first lesion during the study without occurrence of CIN2+ within 60 days of first treatment.

³One woman in vaccine group and 9 women in control group had CIN2+ 60 days or more after first treatment.

⁴Women who were DNA negative for all 14 of the oncogenic HPV types investigated, seronegative for HPV-16 and HPV-18, and had normal cytology at baseline.

⁵HPV DNA positive by PCR.

Abbreviations: AGC: atypical glandular cells; ASC-H: atypical squamous cells, cannot exclude HSIL; ASC-US: atypical squamous cells of undetermined significance; CIN2+: cervical intraepithelial neoplasia grade 2 or greater; HPV: human papillomavirus; HSIL: high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion; n (%): number (percentage) of subjects in given category; TVC: total vaccinated cohort.

Table 3. Vaccine efficacy against subsequent histopathologically confirmed disease and cytological abnormalities in women who underwent surgical treatment for a first lesion during the study

Endpoint	Interval since surgery for first lesion	HPV type in lesion	Group	N	Cases	Rate (95% CI) ¹	Efficacy (95% CI)
CIN2+	≥60 days	Irrespective of HPV DNA	Vaccine	190	1	0.24 (0.01–1.32)	88.2% (14.8 to 99.7)
			Control	264	9	2.01 (0.92–3.81)	
		HPV-16/18	Vaccine	190	0	0.00 (0.00–0.87)	100% (–63.1 to 100)
			Control	265	4	0.87 (0.24–2.24)	
CIN1+	≥60 days	Irrespective of HPV DNA	Vaccine	190	12	2.91 (1.50–5.08)	42.6% (–21.1 to 74.1)
			Control	264	22	5.07 (3.18–7.68)	
		HPV-16/18	Vaccine	190	0	0.00 (0.00–0.87)	100% (26.1 to 100)
			Control	265	7	1.55 (0.62–3.19)	
LSIL	≥60 days	Irrespective of HPV DNA	Vaccine	101	27	13.40 (8.83–19.50)	–30.5% (–142.7 to 29.0)
			Control	110	21	10.27 (6.36–15.70)	
		HPV-16/18	Vaccine	160	1	0.29 (0.01–1.61)	89.5% (21.6 to 99.8)
			Control	163	8	2.75 (1.19–5.41)	
HSIL	≥60 days	Irrespective of HPV DNA	Vaccine	159	0	0.00 (0.00–1.04)	100% (–59.4 to 100)
			Control	215	4	1.07 (0.29–2.74)	
		HPV-16/18	Vaccine	174	0	0.00 (0.00–0.95)	100% (–3950.4 to 100)
			Control	234	1	0.25 (0.01–1.38)	

¹Incidence rate of women reporting at least one event per 100-person years.

Abbreviations: CI: confidence interval; CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus; HSIL: high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion. N: number of women in each group who underwent surgery for a first cervical lesion and who did not have the specified event within 60 days after treatment of the first cervical lesion. Cases: number of women with at least one event at least 60 days after treatment for a first cervical lesion.

vaccine and control groups, respectively) and 54.5% (–42.0 to 87.6) for VIN/VaIN2+ (five and 11 cases, respectively). The number of women in vaccine and control groups with external genital lesions 60 days or more after surgical therapy, regardless of HPV DNA, was seven vs. four for VIN/VaIN1+ and one vs. one for VIN/VaIN2+, respectively. These were all VaIN lesions and no VIN was reported. The one subject in the vaccine group classified as having VaIN2 after surgery had VaIN3 (HPV-68 DNA positive) and CIN2 (HPV-39 DNA positive; Case 1: Table 4; Fig. 2).

In sensitivity analyses in which case counting started 30, 90 or 120 days post-surgery, estimates of vaccine efficacy were generally similar to when counting started 60 days post-surgery (Supporting Information Table S1).

Discussion

In this *post-hoc* analysis, we show that women who undergo surgical therapy after vaccination with the HPV-16/18 vaccine may continue to benefit due to a reduction in the risk of

developing new or recurrent CIN2+. In the vaccine group, women who had been diagnosed and treated for a first cervical lesion were protected against subsequent CIN2+ associated with HPV-16 and/or HPV-18, with no new cases detected. However, there was an effect over and above protection against vaccine types, with efficacy of 88% against subsequent CIN2+, regardless of causal HPV type. The HPV-16/18 vaccine has consistently shown cross-protective efficacy against certain non-vaccine oncogenic HPV types (i.e., HPV-31, –33, –45 and –51),⁷ and thus cross-protection is likely to contribute to the high efficacy observed in our analysis.

We did not show vaccine efficacy against subsequent CIN1+ irrespective of HPV type, as the majority of low-grade lesions detected after surgical therapy were associated with non-vaccine HPV types, but we did show significant efficacy against CIN1+ associated with vaccine HPV types. The vaccine was not efficacious in preventing subsequent LSIL irrespective of HPV type, but significantly reduced LSIL

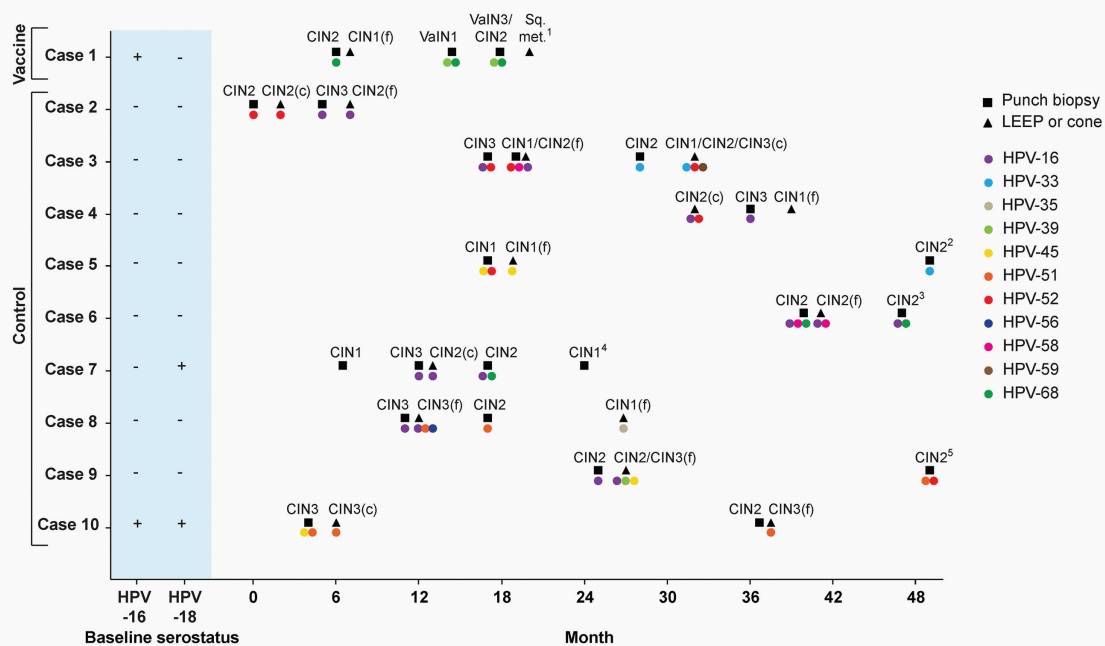


Figure 2. Biopsy type, histopathological diagnosis and oncogenic HPV DNA in lesion for women who had CIN2+ at least 60 days after surgical therapy for cervical disease. ¹Case 1: no high-risk HPV DNA detected in LEEP biopsy at month 20. Exit colposcopy at month 60 was normal. ²Case 5: cone treatment was not needed at month 49 and follow-up cytology was normal. ³Case 6: subject was followed up according to local practice and colposcopy was done approximately 2 weeks later (outside of study). ⁴Case 7: colposcopy at months 30 and 48 were negative and no further treatment was done. ⁵Case 9: no further follow-up information is available after month 49. Abbreviations: CIN: cervical intraepithelial neoplasia; f: disease-free margins; c: compromised margins; Sq.met.: squamous metaplasia; VaIN: vaginal intraepithelial neoplasia; LEEP: loop electrosurgical excision procedure.

associated with HPV-16 and/or HPV-18. It has previously been documented that many low-grade cervical lesions will regress spontaneously without intervention,²³ whereas HPV-16 and HPV-18 infections have a propensity for persistence and progression to CIN2+ compared with some other oncogenic HPV types.^{24,25}

Our results are generally in line with data published for the licensed quadrivalent HPV-6/11/16/18 vaccine (*Gardasil*, Merck & Co), which, in a *post-hoc* analysis similar to the one we conducted, was shown to reduce the frequency of subsequent CIN2+ 60 days or more after surgery irrespective of HPV type by 65% (20 to 86%).²² Additionally, a recent prospective, nonrandomized study conducted in Korea showed that administration of the quadrivalent HPV vaccine commencing one week after treatment by LEEP for CIN2+ may prevent disease recurrence, with significantly fewer vaccinated than nonvaccinated women having recurrence of CIN2+ (2.5% [9/360] vs. 7.2% [27/377], $p < 0.01$).²⁶

Rates of recurrence of histologically proven CIN2+ after treatment for a previous high-grade cervical lesion vary from center to center and are influenced by a number of factors including initial diagnosis, age, treatment type and duration of follow-up. However, the rate of 3.4% (9 of 264 women) observed in the control group of PATRICIA is broadly similar to recurrence rates observed in previous studies.^{17,22,27,28} We found that most women who developed CIN2+ post-

treatment in our cohort (and in a similar evaluation of the quadrivalent HPV-6/11/16/18 vaccine²²) had evidence of exposure to high-risk HPV at baseline before vaccination.

The precise mechanism of action for protection against subsequent cervical lesions in women who have had surgery for a first lesion is not known. The vaccine would be expected to provide protection against *de novo* HPV infection with vaccine HPV types and some cross-protection against non-vaccine HPV types. An additional potential mechanism is that for women who had been previously infected with HPV (with naturally acquired immunity, but with no HPV DNA detected), boosting the natural immune response by vaccination may keep the virus in check, preventing it from becoming an active productive viral infection with subsequent lesion development. Support for this theory comes from Phase III vaccine trials, which show that women who had no HPV DNA detected, but who had a naturally acquired serological response, were less likely to develop lesions than those who were DNA and antibody-positive.^{29,30}

It should be recognized that there is a difference between the incidence and regression rates of HPV and/or CIN in young women as compared to women older than 25 years age. Therefore, while the biology of HPV and CIN lesions is identical irrespective of age, there is a difference in the natural history for outcomes with respect to age. This is well exemplified by the Costa Rican natural history study, where

Table 4. Clinical and virological characteristics for women who had CIN2 + 60 days or more after surgical therapy for cervical disease

Case (Age)	Baseline serostatus		Cytology		First lesion		Second lesion	
	HPV-16	HPV-18	Time	Cytology	HPV DNA	Time/biopsy or treatment/diagnosis (high risk HPV DNA by PCR)	Time/biopsy or treatment/diagnosis (high risk HPV DNA by PCR)	Time/biopsy or treatment/diagnosis (high risk HPV DNA by PCR)
Vaccine group								
1	Positive	Negative	M0	LSIL	HPV-16			
(16 y)			M6	HSIL	HPV-68	M6/punch/CIN2 (HPV-68)		
			M12	LSIL	HPV-68	M8/cone/CIN1 (disease-free margins) (no high risk HPV type detected)	M14/punch/VaIN1 (HPV-39/68)	
			M18	HSIL	HPV-39/52/68		M19/punch/VaIN3 (HPV-68)	
							M19/punch/CIN2 (HPV-39)	
							M20/LEEP/Squamous metaplasia (no high risk HPV type detected). No treatment was done.	
			M24	Normal	None			
			M30	Normal	HPV-52			
			M36	Normal	None			
			M48	ASC-US P	HPV-51/68			M60 (exit colposcopy)/no abnormality detected
Control group								
2	Negative	Negative	M0	ASC-H	HPV-51/52	M0/punch/CIN2 (HPV-52)		
(17 y)			M6	LSIL	HPV-16	M2/LEEP/CIN2 (compromised margins) (HPV-52)	M5/punch/CIN3 (HPV-16)	
			M12	Normal	HPV-16/52/56		M7/LEEP/CIN2 (disease-free margins) (HPV-16)	
			M18	Normal	HPV-31			
			M24	Normal	HPV-31			
			M30	Normal	None			
			M36	Normal	None			
			M48	Normal	None			
3	Negative	Negative	M0	Normal	HPV-31/52			
(18 y)			M18	HSIL	HPV-16/52	M17/punch/CIN3 (HPV-16/52)		
			M24	LSIL	HPV-33/52/68	M19/punch/CIN1 (HPV-52/58)	M28/punch/CIN2 (HPV-33)	
			M30	Missing	HPV-33	M19/LEEP/CIN2 (disease-free margins) (HPV-16)	M32/LEEP/CIN1 (HPV-33/52)	
							M32/LEEP/CIN2 (HPV33/59)	

Table 4. Clinical and virological characteristics for women who had CIN2+ 60 days or more after surgical therapy for cervical disease (Continued)

Case (Age)	Baseline serostatus		Cytology		First lesion		Second lesion	
	HPV-16	HPV-18	Time	Cytology	HPV DNA	Time/biopsy or treatment/diagnosis (high risk HPV DNA by PCR)	Time/biopsy or treatment/diagnosis (high risk HPV DNA by PCR)	Time/biopsy or treatment/diagnosis (high risk HPV DNA by PCR)
4 (19 y)			M36	Normal	HPV-59		M32/LEEP/CIN3 (compromised margins) (HPV-33)	
			M42	Normal	HPV-59			
			M48	ASC-US N	HPV-33/52			
	Negative	Negative	M0	LSIL	HPV-16			
			M12	Normal	HPV-16			
			M18	Missing	HPV-16			
			M24	Normal	HPV-16/52			
			M30	HSIL	HPV-16/52	M31/LEEP/CIN2 (compromised margins) (HPV-16/52)		
			M36	HSIL	HPV-16		M36/punch/CIN3 (HPV-16)	
			M48	Normal	None		M39/cone/CIN1 (disease-free margins) (no high risk HPV type detected)	
5 (24 y)	Negative	Negative	M0	Normal	None			
			M6	Missing	HPV-45/52			
			M12	LSIL	HPV-45/52			
			M18	HSIL	HPV-45/52	M16/punch/CIN1 (HPV-45/52)		
			M24	Normal	None	M19/cone/CIN1 (disease-free margins) (HPV-45)		
			M30	Normal	HPV-33			
			M36	ASC-US P	HPV-33			
			M42	LSIL	HPV-33		M49/punch/CIN3 (HPV-33)	
			M48	LSIL	HPV-33		Cone treatment was not needed. Follow-up cytology was normal.	
	6 (16 y)	Negative	Negative	M0	Normal	None		
			M6	Missing	HPV-51			
			M12	Normal	HPV-51			
			M18	Missing	HPV-51			
			M24	Normal	None			
			M30	Missing	HPV-58			
			M36	ASC-US P	HPV-16/45/58	M40/punch/CIN2 (HPV-16/58/68)		

Table 4. Clinical and virological characteristics for women who had CIN2+ 60 days or more after surgical therapy for cervical disease (Continued)

Case (Age)	Baseline serostatus		Cytology		First lesion		Second lesion	
	HPV-16	HPV-18	Time	Cytology	HPV DNA	Time/biopsy or treatment/diagnosis (high risk HPV DNA by PCR)	Time/biopsy or treatment/diagnosis (high risk HPV DNA by PCR)	Second lesion
7 (17 y)	Negative	Positive	M0	LSIL	HPV-16/51/66	M41/LEEP/CIN2 (disease-free margins) (HPV-16/58)	M47/punch/CIN2 (HPV-16/68)	Followed up according to local practice. Colposcopy was done approximately 2 weeks later (outside of the study).
			M6	ASC-US P	HPV-16/33/51/66	M6/punch/CIN1 (no high risk HPV type detected)		
			M12	LSIL	HPV-16/33/51	M12/punch/CIN3 (HPV-16)		
			M18	LSIL	HPV-16/56/68	M13/LEEP/CIN2 (compromised margins) (HPV-16)	M17/punch/CIN2 (HPV-16/68)	
			M24	ASC-US P	HPV-16/56		M24/punch/CIN1 (no high risk HPV type detected)	
			M30	ASC-US P	HPV-56		M30/colposcopy was negative and no treatment was done	
			M36	Normal	HPV-16/68			
			M42	Normal	HPV-16/56/68			
			M48	LSIL	HPV-35/66		M48/exit colposcopy was negative and no treatment was done	
	8 (19 y)	Negative	Negative	M0	ASC-US N	None		
			M6	Missing	HPV-16/51	M11/punch/CIN3 (HPV-16)		
			M12	HSIL	HPV-16/51	M12/LEEP/CIN3 (HPV-16/56) and CIN3 (disease-free margins) (HPV-16/51)	M17/punch/CIN2 (HPV-51)	
			M18	LSIL	HPV-16/51			
			M24	LSIL	HPV-35		M27/LEEP/CIN1 (disease-free margins) (HPV-35)	
			M30	Normal	HPV-39			
			M36	Normal	None			
			M42	Normal	HPV-39			
			M48	ASC-US N	HPV-39/52			
9		Negative	Negative	M0	Normal	HPV-52		

Table 4. Clinical and virological characteristics for women who had CIN2 + 60 days or more after surgical therapy for cervical disease (Continued)

Baseline serostatus			Cytology		First lesion		Second lesion	
Case (Age)	HPV-16	HPV-18	Time	Cytology	HPV DNA	Time/biopsy or treatment/diagnosis (high risk HPV DNA by PCR)	Time/biopsy or treatment/diagnosis (high risk HPV DNA by PCR)	
(16 y)			M6	Missing	None			
			M12	LSIL	HPV-39/58			
			M18	LSIL	HPV-16/56/58			
			M24	LSIL	HPV-16/39/66	M25/punch/CIN2 (HPV-16)		
			M30	Normal	HPV-68	M27/LEEP/CIN2 (HPV-16/39) and CIN3 (disease-free margins) (HPV-16/45)		
			M36	ASC-US P	HPV-51/68			
			M42	LSIL	HPV-51/68		M49/punch/CIN2 (HPV-51/52)	
								Subject was not treated within the study. No further follow-up information available.
			M48	Normal	HPV-51			
10	Positive	Positive	M0	HSIL	HPV-51/66	M4/punch/CIN3 (HPV-45/51)		
(25 y)			M6	Missing	HPV-45/51	M6/LEEP/CIN3 (compromised margins) (HPV-51)		
			M12	ASC-US P	HPV-45/51			
			M18	Missing	HPV-51			
			M24	LSIL	HPV-51			
			M30	Missing	HPV-51			
			M36	HSIL	HPV-51		M37/Punch/CIN2 (no high risk HPV type detected)	
			M42	Normal	None		M37/Curettage/CIN1 (no high risk HPV type detected)	
			M48	Normal	None		M37/LEEP/CIN3 (disease-free margins) (HPV-51)	

Abbreviations: ASC-H: atypical squamous cells cannot exclude high-grade squamous epithelial lesion; ASC-US: atypical squamous cells of undetermined significance; CIN: cervical intraepithelial neoplasia; HSIL: high-grade squamous intraepithelial lesion; LEEP: loop electrosurgical excision procedure; LSIL: low-grade squamous intraepithelial lesion; M: month; N: negative for oncogenic HPV DNA by hybrid capture II; P: positive for oncogenic HPV DNA by hybrid capture II; y: years.

they found that the rate of new HPV infections declined with age.³¹ Interim results from an ongoing study of the HPV-16/18 AS04-adjuvanted vaccine in women older than 25 years showed that vaccine efficacy was similar in women who were seropositive and DNA-negative at baseline compared with those who were both DNA-negative and seronegative.³² The risk however of progression of HPV infection to CIN2+ in women >25 years in this study was similar to that in women 15–25 years in PATRICIA.³³

The mechanism may relate to vaccine boosting of cellular adaptive and innate immune responses, as shown by the efficacy of the HPV-16/18 vaccine against genital warts primarily due to HPV-6/11,³⁴ and recurrent respiratory papillomatosis due to HPV-6/11 (AM Kaufmann, personal communication, 2013). Vaccination induces a strong TH1 helper T-cell response against the vaccine L1 antigen that is cross-reactive with non-vaccine type L1. T-cells are also induced to other HPV antigens such as E6, supporting reversal of tolerance and kick starting a broad immune response. In addition, previous studies reported therapeutic vaccination as an excellent method to stimulate the immune system. In a phase I/II clinical trial evaluating the use of MVA E2 recombinant vaccinia virus in treating CIN1, CIN2 and CIN3 lesions associated with HPV infection, cells cytotoxic to HPV-transformed cells, and the generation of antibodies against MVA E2, correlated with the regression of lesions and reduction of HPV viral load in all MVA E2-treated patients.³⁵ A recent study, the first therapeutic vaccine, VGX-3100 composed of synthetic plasmids targeting HPV-16 and HPV-18 E6 and E7 proteins, is encouraging, but has not yet shown efficacy against CIN2/3 associated with HPV-16 and HPV-18.³⁶ The mechanism of action of the vaccine does not only involve antibodies, but also cell-mediated immunity. A strength of our study is that we have detailed information on margin status of excisional material for each woman treated for a first cervical lesion who subsequently developed lesions post-surgery. Three of the four women with compromised margins had subsequent cervical lesions associated with at least one of the HPV genotypes found in the original lesion, suggesting residual disease.

However, our analysis has some limitations. PATRICIA was not designed to evaluate the effects of vaccination post-treatment and this was a *post-hoc* analysis. Women with a prior history of colposcopy were excluded from PATRICIA, so we were unable to evaluate vaccine efficacy in women who underwent treatment for HPV-related disease prior to vaccination. As a surrogate, we identified women who received surgery for a first cervical lesion during the study and investigated the impact of vaccination on any subsequent lesions postsurgery, but the subset of women who underwent surgery was not a randomized group. Due to the efficacy of the vaccine in preventing a first occurrence of cervical disease, more subjects were referred for colposcopy and treatment in the control group than the vaccine group, which introduced bias into the analysis. The two groups were not necessarily

comparable for baseline characteristics as women in the vaccine group would be expected to have fewer lesions associated with HPV types –16 and –18. Furthermore, due to the relatively small number of women who had surgical therapy and then subsequently experienced new or recurrent disease, our analysis has limited statistical power. We were unable to reliably estimate vaccine efficacy against subsequent VIN/VaIN due to the small number of observed cases. Visual inspection of the vagina and vulva was introduced *via* a protocol amendment late in the study and external genital lesions did not need to be biopsied.

Finally, women included in this study were younger than those included in most screening programs today (≥ 20 years of age). While current vaccination programs aim primarily at adolescent girls and young women, older women are typically offered screening and cytology. As noted for those naïve to vaccine-related HPV infections, vaccine efficacy is not age dependent. Studies of HPV vaccination in women aged up to 55 years have shown a protection of ~90% against HPV-16/18 infections for those naïve to these infections. Based on this, the recently published HPV-FASTER concept considers expanding routine vaccination programmes to women of up to 45 years of age, along with at least one HPV DNA-screening tests at the age of 30.³⁷ Vaccination in older women might not be cost-efficient until current vaccine prices decline substantially. However, expanding the indications for HPV vaccination and adapting HPV screening programs among older women could potentially reduce cervical cancer incidence, and decrease the burden to health-care systems more quickly, particularly in countries from Central and Eastern Europe, Latin America, Asia and some more-developed parts of Africa where screening is nonexistent or not effective.

An adequately powered, randomized, double-blind, placebo-controlled trial, delivering the vaccine or control after treatment for CIN3, would ideally be needed to overcome the above-mentioned potential biases and to estimate the true efficacy of HPV vaccination after treatment in preventing recurrence of high-grade cervical disease. Trials on CIN3+ may be not feasible given the number of subjects to complete, but those countries with high coverage of vaccines and good surveillance (comprehensive cytology and/HPV DNA registries) should be able to answer these questions with time.^{22,28}

When unvaccinated women present for colposcopy and/or treatment, gynaecologists should actively seek to vaccinate them. Indeed, all sexually active adult women should be encouraged to have the vaccine regardless of whether they have had a cervical abnormality as they can benefit, with the proviso that such women will continue to need active screening (HPV DNA or cytologically).^{38–40} Reducing the incidence of recurrent and/or residual lesions in women who have undergone ablative or excisional treatment would be expected to lead to fewer repeat treatments and associated hospital

visits and a reduction in the negative psychological sequelae and potential obstetric consequences of repeated treatment.

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Contributors

GSK designed the study in collaboration with investigators and coordinated collection, analysis and interpretation of data. Investigators from the HPV PATRICIA Study Group collected data for the trial and cared for the participants. All authors contributed to study design, acquisition of data or statistical analyses and interpretation of the data. All authors had full access to all the trial data, reviewed and commented on a draft of the manuscript and had final responsibility for the decision to submit for publication. The manuscript was developed and coordinated by the authors in collaboration with an independent medical writer and a publication manager, both working on behalf of GSK.

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Cervarix is a registered trade mark of the GSK group of companies.

Competing Interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that: GC, DD and FS are employees of the GSK group of companies. DD and FS hold shares in the GSK group of companies as part of their employee remuneration. GD was a full time employee of the GSK group of companies at the time the study was conducted. GD owns stock shares and options of the GSK group of

companies as well as several patents in the HPV field. GD is currently employed by Takeda Pharmaceuticals. DA reports grants from the GSK group of companies for the conduct of HPV vaccination studies. The institution of JCT received grants from the GSK group of companies to conduct this study. JCT also received nonfinancial support and personal fees from the GSK group of companies during the conduct of this study and outside the submitted work. ML reports grants from the GSK group of companies and Merck & Co. Inc. through his institution during the conduct of this study. NSDC received funding through his institution from the GSK group of companies to conduct HPV vaccine trials. NSDC also received payment from the GSK group of companies for lectures and participation in advisory board. PN reports funding from the GSK group of companies through his institution for his participation in the development studies of the HPV vaccine. SRS is an investigator on the PATRICIA trial and her institution received funds from the GSK group of companies to reimburse costs associated with the collection of data for this trial. GSK Australia and Seqirus provided funds to the institution of SRS for educational research relating to HPV and for research evaluating Australia's HPV vaccination program, respectively. As an investigator, SRS also received travel reimbursement from the GSK group of companies and honorarium for attendance at Global Advisory Boards. MRDRR received honoraria, travel support and payment for lecture including speakers bureaus from the GSK group of companies outside of the submitted work. SMG received through her institution grants from the GSK group of companies and Merck & Co. Inc. to conduct phase 3 clinical HPV trials and from CSL Bio. SMG reports fares and accommodation reimbursements to participate in Merck advisory boards. SMG also received honoraria for lectures and work performed in her own time. TFS received honoraria for lecturing, member of advisory boards and conducting clinical trials for the GSK group of companies. XB reports institutional research grants on vaccine trials from the GSK group of companies, Sanofi Pasteur MSD and Merck & Co. Inc. XB also received educational and travel grants from the GSK group of companies, Sanofi Pasteur MSD and Merck & Co. Inc. XB is an advisory board member of Sanofi Pasteur MSD and Merck & Co. Inc. UJ received funding through his institution from the GSK group of companies to do HPV vaccine studies. UJ also received travel reimbursement from the GSK group of companies. XC reports grants, personal fees, and non-financial support from the GSK group of companies for attending speakers bureau and scientific meetings. XC also reports grants and personal fees from Sanofi Pasteur MSD and Merck & Co. Inc. S-NC, MJVG, JH, GL, JP, KP, WAJP and JS declare that they have no conflicts of interest.

References

- Walboomers JM, Jacobs MV, Manos MM, *et al.* Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999; 189:12–9.
- Li N, Franceschi S, Howell-Jones R, *et al.* Human papillomavirus type distribution in 30,848

- invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication. *Int J Cancer* 2010;128:927–35.
3. de Sanjosé S, Quint WG, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol* 2010;11:1048–56.
 4. Paavonen J, Jenkins D, Bosch FX, et al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. *Lancet* 2007;369:2161–70.
 5. Paavonen J, Naud P, Salmemon J, et al. Efficacy of human papillomavirus (HPV)–16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. *Lancet* 2009;374:301–14.
 6. Lehtinen M, Paavonen J, Wheeler CM, et al. Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *Lancet Oncol* 2012;13:89–99.
 7. Wheeler CM, Castellsague X, Garland SM, et al. Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *Lancet Oncol* 2012;13:100–10.
 8. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N Engl J Med* 2007;356:1928–43.
 9. FUTURE II Study Group. Prophylactic efficacy of a quadrivalent human papillomavirus (HPV) vaccine in women with virological evidence of HPV infection. *J Infect Dis* 2007;196:1438–46.
 10. Ault KA, FUTURE II Study Group. Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3, and adenocarcinoma in situ: a combined analysis of four randomised clinical trials. *Lancet* 2007;369:1861–68.
 11. Muñoz N, Kjaer SK, Sigurdsson K, et al. Impact of human papillomavirus (HPV)–6/11/16/18 vaccine on all HPV-associated genital diseases in young women. *J Natl Cancer Inst* 2010;102:325–39.
 12. Hildesheim A, Herrero R, Wacholder S, et al. Effect of human papillomavirus 16/18 L1 virus-like particle vaccine among young women with preexisting infection: a randomized trial. *Jama* 2007;298:743–53.
 13. Pettersson F, Walker B. Invasive carcinoma of the uterine cervix following diagnosis and treatment of in situ carcinoma. Record linkage study within a National Cancer Registry. *Radiother Oncol* 1989;16:115–20.
 14. Kalliala I, Anttila A, Pukkala E, et al. Risk of cervical and other cancers after treatment of cervical intraepithelial neoplasia: retrospective cohort study. *BMJ* 2005;331:1183–85.
 15. Soutter WP, Sasieni P, Panoskaltis T. Long-term risk of invasive cervical cancer after treatment of squamous cervical intraepithelial neoplasia. *Int J Cancer* 2006;118:2048–55.
 16. Strander B, Andersson-Ellstrom A, Milsom I, et al. Long term risk of invasive cancer after treatment for cervical intraepithelial neoplasia grade 3: population based cohort study. *BMJ* 2007;335:1077.
 17. Melnikow J, McGahan C, Sawaya GF, et al. Cervical intraepithelial neoplasia outcomes after treatment: long-term follow-up from the British Columbia Cohort Study. *J Natl Cancer Inst* 2009;101:721–28.
 18. Rebolj M, Helmerhorst T, Habbema D, et al. Risk of cervical cancer after completed post-treatment follow-up of cervical intraepithelial neoplasia: population based cohort study. *BMJ* 2012;345:e6855.
 19. van Doorn LJ, Molijn A, Kleter B, et al. Highly effective detection of human papillomavirus 16 and 18 DNA by a testing algorithm combining broad-spectrum and type-specific PCR. *J Clin Microbiol* 2006;44:3292–98.
 20. Pagliusi SR, Aguado MT. Efficacy and other milestones for human papillomavirus vaccine introduction. *Vaccine* 2004;23:569–78.
 21. Castle PE, Schiffman M, Wheeler CM, et al. Evidence for frequent regression of cervical intraepithelial neoplasia-grade 2. *Obstet Gynecol* 2009;113:18–25.
 22. Joura EA, Garland SM, Paavonen J, et al. Effect of the human papillomavirus (HPV) quadrivalent vaccine in a subgroup of women with cervical and vulvar disease: retrospective pooled analysis of trial data. *BMJ* 2012;344:e1401.
 23. Holowaty P, Miller AB, Rohan T, et al. Natural history of dysplasia of the uterine cervix. *J Natl Cancer Inst* 1999;91:252–58.
 24. Khan MJ, Castle PE, Lortincz AT, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J Natl Cancer Inst* 2005;97:1072–79.
 25. Guan P, Howell-Jones R, Li N, et al. Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. *Int J Cancer* 2012;131:2349–59.
 26. Kang WD, Choi HS, Kim SM. Is vaccination with quadrivalent HPV vaccine after loop electro-surgical excision procedure effective in preventing recurrence in patients with high-grade cervical intraepithelial neoplasia (CIN2-3)? *Gynecol Oncol* 2013;130:264–68.
 27. Chan BK, Melnikow J, Slee CA, et al. Posttreatment human papillomavirus testing for recurrent cervical intraepithelial neoplasia: a systematic review. *Am J Obstet Gynecol* 2009;200:422 e1–9.
 28. Tan JH, Garland SM, Tabrizi SN, et al. Hybrid Capture II testing for high-risk human papillomavirus DNA in the follow-up of women treated for high-grade cervical intraepithelial neoplasia. *J Low Genit Tract Dis* 2013;17:308–14.
 29. Szarewski A, Poppe WA, Skinner SR, et al. Efficacy of the human papillomavirus (HPV)–16/18 AS04-adjuvanted vaccine in women aged 15–25 years with and without serological evidence of previous exposure to HPV-16/18. *Int J Cancer* 2012;131:106–16.
 30. Olsson SE, Kjaer SK, Sigurdsson K, et al. 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. *Hum Vaccin* 2009;5:696–704.
 31. Rodríguez AC, Schiffman M, Herrero R, et al. Low risk of type-specific carcinogenic HPV re-appearance with subsequent cervical intraepithelial neoplasia grade 2/3. *Int J Cancer* 2012;131:1874–81. doi: 10.1002/ijc.27418
 32. Skinner SR, Szarewski A, Romanowski B, et al. 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. *Lancet* 2014;384:2213–27.
 33. Skinner SR, Wheeler CM, Romanowski B, et al. Progression of HPV infection to detectable cervical lesions or clearance in adult women: analysis of the control arm of the VIVIANE study. *Int J Cancer* 2016;138:2428–38. doi: 10.1002/ijc.29971.
 34. Szarewski A, Skinner SR, Garland SM, et al. Efficacy of the HPV-16/18 AS04-adjuvanted vaccine against low-risk HPV types (PATRICIA randomized trial): an unexpected observation. *J Infect Dis* 2013;208:1391–96.
 35. Corona Gutierrez CM, Tinoco A, Navarro T, et al. Therapeutic vaccination with MVA E2 can eliminate precancerous lesions (CIN 1, CIN 2, and CIN 3) associated with infection by oncogenic human papillomavirus. *Hum. Gene Ther* 2004;15:421–31.
 36. Trimble CL, Morrow MP, Kravnyak KA, et al. Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial. *Lancet* 2015;386:2078–88.
 37. Bosch FX, Robles C, Díaz M, et al. HPV-FASTER: broadening the scope for prevention of HPV-related cancer. *Nat Rev Clin Oncol* 2016;13:119–32. doi: 10.1038/nrclinonc.2015.146.
 38. Castle PE, Schmeler KM. HPV vaccination: for women of all ages? *Lancet* 2014;384:2178–80.
 39. Franceschi S, Denny L, Irwin KL, et al. EUROGIN 2010 roadmap on cervical cancer prevention. *Int J Cancer* 2011;128:2765–74.
 40. Castellsagué X, Bruni L, de Sanjosé S, et al. Other potential indications and targets for HPV vaccination: the case of mid-adult women. EUROGIN Congress 2013, Florence, Italy; Nov 3–6, 2013. Report No SS 23-4 (abstr). Available at: <http://www.eurogin.com/2013/images/pdf/EUROGIN-2013-Abstracts-Part-1.pdf>. Accessed 11 November 2014.