# 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

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## Summary

**Background** The aim of this interim analysis of a large, international phase III study was to assess the efficacy of an AS04 adjuvanted L1 virus-like-particle prophylactic candidate vaccine against infection with human papillomavirus (HPV) types 16 and 18 in young women.

**Methods** 18 644 women aged 15–25 years were randomly assigned to receive either HPV16/18 vaccine (n=9319) or hepatitis A vaccine (n=9325) at 0, 1, and 6 months. Of these women, 88 were excluded because of high-grade cytology and 31 for missing cytology results. Thus, 9258 women received the HPV16/18 vaccine and 9267 received the control vaccine in the total vaccinated cohort for efficacy, which included women who had prevalent oncogenic HPV infections, often with several HPV types, as well as low-grade cytological abnormalities at study entry and who received at least one vaccine dose. We assessed cervical cytology and subsequent biopsy for 14 oncogenic HPV types by PCR. The primary endpoint—vaccine efficacy against cervical intraepithelial neoplasia (CIN) 2+ associated with HPV16 or HPV18—was assessed in women who were seronegative and DNA negative for the corresponding vaccine type at baseline (month 0) and allowed inclusion of lesions with several oncogenic HPV types. This interim event-defined analysis was triggered when at least 23 cases of CIN2+ with HPV16 or HPV18 DNA in the lesion were detected in the total vaccinated cohort for efficacy. Analyses were done on a modified intention-to-treat basis. This trial is registered with the US National Institutes of Health clinical trial registry, number NCT00122681.

**Findings** Mean length of follow-up for women in the primary analysis for efficacy at the time of the interim analysis was 14·8 (SD 4·9) months. Two cases of CIN2+ associated with HPV16 or HPV18 DNA were seen in the HPV16/18 vaccine group; 21 were recorded in the control group. Of the 23 cases, 14 (two in the HPV16/18 vaccine group, 12 in the control group) contained several oncogenic HPV types. Vaccine efficacy against CIN2+ containing HPV16/18 DNA was 90·4% (97·9% CI 53·4–99·3; p<0·0001). No clinically meaningful differences were noted in safety outcomes between the study groups.

**Interpretation** The adjuvanted HPV16/18 vaccine showed prophylactic efficacy against CIN2+ associated with HPV16 or HPV18 and thus could be used for cervical cancer prevention.

## Introduction

The necessary role of oncogenic human papillomavirus (HPV) infection in cervical cancer provides an opportunity to reduce disease burden through prophylactic vaccination.[1–3] HPV types 16 and 18 account for 70% or more of cases of cervical cancer worldwide.[4] Up to 15 oncogenic HPV types contribute to cervical cancer and several are members of either the A7 (HPV18, 39, 45, 59, 68, 70, and 85) or A9 (HPV16, 31, 33, 35, 52, 58, and 67) papillomavirus species.[5,6]

An HPV16/18 L1 virus-like-particle candidate vaccine (GlaxoSmithKline Biologicals, Rixensart, Belgium), adjuvanted with 3-*O*-desacyl-4′-monophosphoryl lipid A and aluminium hydroxide (AS04), has shown complete prevention of 12-month persistent infections with the combined endpoint of HPV16 infection, HPV18 infection, or HPV16 and HPV18 co-infection (HPV16/18), and associated combined cervical intraepithelial neoplasia (CIN grades 1, 2, and 3) in fully vaccinated young women who were seronegative for HPV16 and HPV18 and negative for any cervical oncogenic HPV DNA at study entry.[7,8] High efficacy has been shown through 4·5 years of follow-up, together with sustained levels of antibodies against HPV16 and HPV18.[8] The vaccine has also shown evidence of cross-protection against incident infection with HPV45 and HPV31, two non-vaccine HPV types that are phylogenetically related to HPV16 and HPV18,[8] which together with HPV16 and HPV18, account for about 80% of cases of cervical cancer worldwide.[4,5]

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The aim of this prespecified interim analysis of a phase III double-blind, randomised controlled trial is to assess the efficacy of this vaccine against CIN2, CIN3, adenocarcinoma in situ, and invasive carcinoma associated with HPV16 or HPV18—a surrogate endpoint for cervical cancer—and against persistent infections with HPV16, HPV18, and other oncogenic HPV types.[9]

## Methods

### Patients

Women were recruited for the PApilloma TRIal against Cancer In young Adults (PATRICIA) study between May, 2004, and June, 2005, in 14 countries (Australia, Belgium, Brazil, Canada, Finland, Germany, Italy, Mexico, Philippines, Spain, Taiwan, Thailand, UK, and USA).

Healthy women aged 15–25 years who reported no more than six lifetime sexual partners before study enrolment (in some countries this criteria was not considered for minors), who agreed to adequate contraception (barrier methods in combination with a spermicide or hormonal contraception) over the vaccination period, and had an intact cervix, were eligible for inclusion. Exclusion criteria were limited to women with a history of colposcopy, who were pregnant or breastfeeding, or who had chronic or autoimmune disease or immunodeficiency.

All participants provided written informed consent, or informed assent with written consent from a parent or legal representative (if below the legal age of consent). All recruitment materials, informed consent/assent forms, protocols, and amendments were approved by independent ethics committees or institutional review boards.

### Procedures

Women were randomised in a 1:1 fashion to receive either the adjuvanted HPV16/18 vaccine or, to provide a health benefit and ensure double-blinding, a control hepatitis A vaccine (investigational formula based on licensed Havrix vaccine;[10] GlaxoSmithKline Biologicals, Rixensart, Belgium) with an internet-based centralised randomisation system. Allocation of treatment numbers was stratified by study site and by age. Because the study is continuing, individual vaccine allocation remains blinded.

Each dose of the HPV16/18 vaccine contained HPV16 and HPV18 L1 virus-like-particle (20 μg of each) adjuvanted with 50 μg 3-O-desacyl-4′-monophosphoryl lipid A and 0·5 mg aluminium hydroxide. Each dose of the control hepatitis A vaccine contained 720 ELISA units (EU) of inactivated hepatitis A antigen and 0·5 mg aluminium hydroxide. The vaccines were identical in appearance, provided in prefilled syringes, and 0·5 mL was administered into the deltoid muscle on a 0, 1, and 6-month schedule.

Women were scheduled to attend a gynaecological examination and routine cervical cytology sampling at months 0 and 12. Cytology was assessed with cervical liquid-based cytology (PreservCyt, Cytyc Corporation, Marlborough, MA, USA).

Clinical management of abnormal cytological results and colposcopy referral was as described previously.[8] Guidelines recommended colposcopy after two consecutive or intermittent reports of oncogenic HPV DNA positive (with Hybrid Capture 2, Gaithersburg, MD, USA) atypical squamous cells of undetermined significance, low-grade squamous cell intraepithelial lesion (independent of HPV DNA results), or one report of atypical glandular cells, high-grade squamous intraepithelial lesion, or atypical squamous cells in which high-grade squamous intraepithelial lesions could not be excluded. The protocol also permitted referral for colposcopy after a single diagnosis of

| | Vaccine group (N=9319) | Control group (N=9325) |
|---|---|---|
| Age (years) | 20·0 (3·1) | 20·0 (3·1) |
| Number of sexual partners in the past 12 months | | |
| None | 294 (4%) | 292 (4%) |
| One | 5861 (74%) | 5868 (74%) |
| Two | 1114 (14%) | 1161 (15%) |
| Three or more | 636 (8%) | 595 (8%) |
| No data | 1414 (..) | 1409 (..) |
| Region | | |
| Asia Pacific | 3173 (34%) | 3180 (34%) |
| Europe | 3224 (35%) | 3221 (35%) |
| Latin America | 1388 (15%) | 1386 (15%) |
| North America | 1534 (16%) | 1538 (16%) |
| Ethnic origin | | |
| Black | 334 (4%) | 358 (4%) |
| White | 5117 (55%) | 5098 (55%) |
| East and southeast Asia | 2173 (23%) | 2173 (23%) |
| Chinese | 761 (8%) | 753 (8%) |
| Hispanic | 668 (7%) | 662 (7%) |
| Other | 266 (3%) | 281 (3%) |
| Compliance with vaccination | | |
| Received only one dose | 295 (3%) | 266 (3%) |
| Received only two doses | 490 (5%) | 487 (5%) |
| Received all three doses | 8534 (92%) | 8572 (92%) |
| Study visit completion | | |
| Attended month 18 study visit | 6566 (70%) | 6480 (69%) |
| Dropouts in total vaccinated cohort* | 496 (5%) | 458 (5%) |
| Non-serious adverse event | 6 (1%) | 3 (0·7%) |
| Serious adverse event | 3 (0·6%) | 5 (1%) |
| Women lost to follow-up | 231 (47%) | 215 (47%) |
| Other | 256 (52%) | 235 (51%) |

Data are mean (SD) or n (%). Percentages do not necessarily add up to 100% because of rounding. *No dropouts were the result of an adverse event related to vaccination; Other includes women who withdrew consent (not due to an adverse event), protocol violation, migrated or moved from study area, personal reasons, unknown reasons, or pregnancy.

*Table 1:* Demographic characteristics of the total vaccinated cohort

oncogenic HPV DNA-positive atypical squamous cells of undetermined significance or a cytological diagnosis of low-grade squamous intraepithelial lesions. For any suspected cervical lesions at colposcopy, biopsy was recommended. CIN2+ (defined histologically as CIN2, CIN3, adenocarcinoma in situ, and invasive carcinoma) was treated by conisation. Study colposcopists were trained every year with standardised sets of colposcopic images to increase uniformity of colposcopic interpretation.

A central laboratory (Quest Diagnostics Clinical Trials, Teterboro, NJ, USA) processed and interpreted results from liquid-based cytology and histology samples. All CIN endpoints were confirmed by an expert histopathology review panel that was blinded to vaccine status, HPV DNA status before biopsy, and cytology reports. HPV DNA testing was done at DDL Diagnostic Laboratory (Voorburg, Netherlands).

Liquid-based cytology samples were collected at months 0, 6, 12, and 18 for HPV DNA testing. A central laboratory assessed 14 oncogenic HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). A validated algorithm for optimum sensitivity in detecting HPV16 or HPV18 DNA was used on the basis of the broad spectrum PCR $SPF_{10}$-$LiPA_{25}$ system (version 1, Innogenetics, Gent, Belgium, manufactured by Labo Bio-medical Products, Rijswijk, Netherlands). If the sample was negative for HPV16 or HPV18 DNA by the $SPF_{10}$-$LiPA_{25}$ system, type-specific PCR for HPV16 or HPV18 was done.[8,11] We defined a positive HPV16 or HPV18 result as detection of either type by $SPF_{10}$-$LiPA_{25}$ system or by type-specific PCR. A sample did not have to be positive for both tests. HPV DNA was examined in microdissected CIN lesions or components of complex lesions of different grades. To exclude laboratory contamination and to determine whether HPV16 or HPV18 DNA could be detected consistently, all biopsies batch processed for PCR with the CIN2+ endpoint biopsies were examined by type-specific HPV16 or HPV18 PCR.

If DNA from several HPV types was detected in a lesion, additional analyses were done to attribute a likely causal association to an HPV type. The attribution of causality was based on the presence of an oncogenic HPV infection preceding the development of CIN. If more than one HPV DNA type was detected in a lesion, the presence of HPV types in one of two immediately preceding cytology sample(s) was considered; where the HPV type present in both the lesion and in one of two immediately preceding cytology sample(s) was the same, this type was considered to be causally associated with that lesion.

To investigate the activity of HPV16 or HPV18 DNA detected in the CIN lesions, we did immunohisto-chemical analyses for HPV16 and HPV18 type-specific E4 gene expression. These exploratory investigations were done on cases in which the causal role of HPV16

| | Vaccine group (N=9319) | Control group (N=9325) |
|---|---|---|
| **Previous and prevalent HPV infection at entry** | | |
| HPV16 | | |
| Seronegative and DNA negative | 7448/9222 (81%) | 7431/9211 (81%) |
| Seropositive and DNA negative | 1258/9222 (14%) | 1302/9211 (14%) |
| Seronegative and DNA positive | 230/9222 (2%) | 228/9211 (2%) |
| Seropositive and DNA positive | 286/9222 (3%) | 250/9211 (3%) |
| Missing data | 97 (..) | 114 (..) |
| HPV18 | | |
| Seronegative and DNA negative | 8035/9235 (87%) | 8058/9242 (87%) |
| Seropositive and DNA negative | 985/9235 (11%) | 968/9242 (10%) |
| Seronegative and DNA positive | 127/9235 (1%) | 114/9242 (1%) |
| Seropositive and DNA positive | 88/9235 (1%) | 102/9242 (1%) |
| Missing data | 84 (..) | 83 (..) |
| **Cytological status at entry\*** | | |
| Women with negative cytology | 8395/9319 (90%) | 8450/9325 (91%) |
| With oncogenic HPV | 1250/8395 (15%) | 1229/8450 (15%) |
| With oncogenic HPV other than vaccine type | 1005/8395 (12%) | 997/8450 (12%) |
| With HPV vaccine type (16/18)† | 413/8395 (5%) | 411/8450 (5%) |
| Women with ASC-US and LSIL | 863/9319 (9%) | 817/9325 (9%) |
| With oncogenic HPV | 607/863 (70%) | 587/817 (72%) |
| With oncogenic HPV other than vaccine type | 523/863 (61%) | 509/817 (62%) |
| With HPV vaccine type (16/18)† | 252/863 (29%) | 213/817 (26%) |
| Women with HSIL, ASC-H, AGC | 45/9319 (0·5%) | 43/9325 (0·5%) |
| With oncogenic HPV | 41/45 (91%) | 39/43 (91%) |
| With oncogenic HPV other than vaccine type | 24/45 (53%) | 30/43 (70%) |
| With HPV vaccine type (16/18)† | 25/45 (56%) | 25/43 (58%) |

Data are n (%). AGC=atypical glandular cells. ASC-H=atypical squamous cells, cannot exclude HSIL. ASC-US=atypical squamous cells of undetermined significance. HSIL=high-grade squamous intraepithelial lesion. LSIL=low-grade squamous intraepithelial lesion. Oncogenic HPV includes HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Oncogenic HPV other than vaccine type includes HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. *16 women in the vaccine group and 15 women in the control group had missing cytology results. †Includes women with HPV16, HPV18, or both.

*Table 2:* **Baseline characteristics in the total vaccinated cohort at study entry**

or HPV18 was in question, due to the detection of several HPV DNA types in the lesions and a lack of detection of HPV16 or HPV18 in any previous samples; representative cases which were clearly assigned to HPV16 or HPV18 were used as positive controls. HPV16 E4 gene product was detected by fluorescence micros-copy with a monoclonal antibody (TVG405) that has been shown to be specific for HPV16 and HPV31.[12] HPV18 E4 gene product was detected with a polyclonal antibody that, under appropriate conditions, is specific for HPV18 and HPV45.[12]

The primary objective was to assess vaccine efficacy against CIN2+ associated with HPV16/18 in women who were seronegative and DNA negative for the corresponding vaccine type at month 0. Association with HPV16/18 was based on DNA detection in the lesion. Secondary objectives included efficacy against CIN1+ (CIN1 and CIN2+) associated with HPV16/18, persistent infection with HPV16/18 (at 6 and 12 months) or other oncogenic HPV types (at 6 months), immunogenicity, and safety.
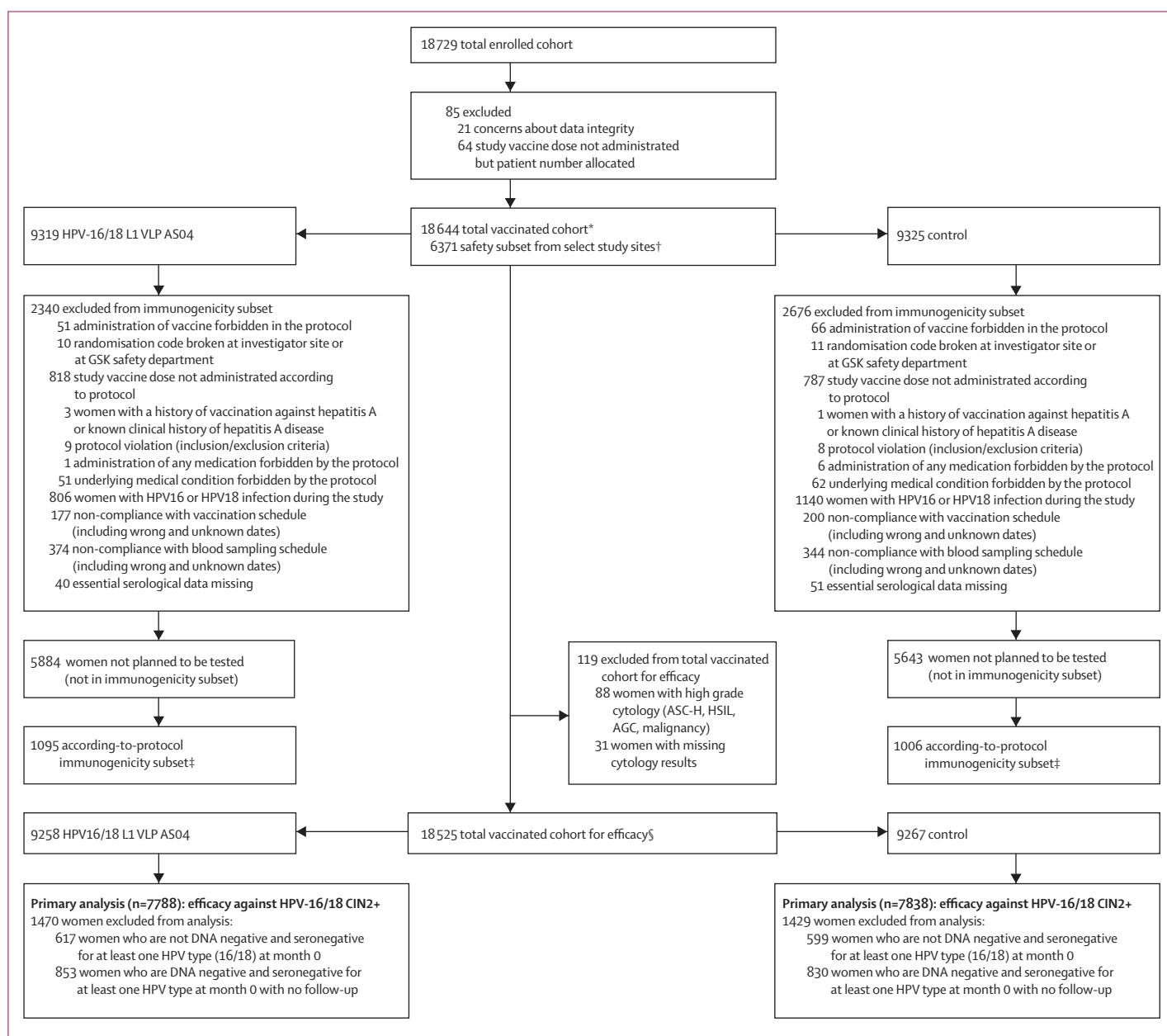
**Figure: Trial profile**

*Includes all women who received at least one vaccine dose. †Safety subset of women from the total vaccinated cohort was assessed for solicited (up to 7 days) and unsolicited adverse events (up to 30 days post-vaccination), no exclusion criteria applied; all women in total vaccinated cohort were assessed for serious adverse events, new onset chronic disease, medically significant conditions, and pregnancies. ‡The according to protocol subset for immunogenicity includes women who met eligibility criteria, complied with protocol procedures, have received all three vaccine doses, and had results for antibodies against at least one vaccine antigen. §Women with normal or low-grade cytology (ie, negative or atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesions) at month 0, who received at least one vaccine dose, and had data available concerning efficacy endpoint were assessed in total vaccinated cohort for efficacy. AGC=atypical glandular cells. ASC-H =atypical squamous cells, cannot exclude HSIL. HSIL=high-grade squamous intraepithelial lesions.

Exploratory objectives included 12-month persistent infection with other oncogenic HPV types. 6-month persistent infection was defined as the detection of DNA from the same HPV type in two consecutive cervical cytology samples collected over any 6-month period, and 12-month persistence as detection of the same HPV type in all available cytology samples collected over any

12-month period. Analyses of persistent infection with HPV16/18 were done in women who were seronegative and DNA negative for the corresponding vaccine type at month 0; analysis of persistent infection with individual HPV types (other than types 16 and 18) were done on women who were DNA negative at month 0 for the corresponding type assessed.

We collected serum samples from women at months 0 and 7 to assess vaccine-induced immune responses to HPV16 or HPV18. In a subset of women from selected study sites (Australia, Brazil, Canada, Finland, Mexico, Thailand, and the USA) serum samples were also collected at months 6 and 12. Antibodies against HPV16 and HPV18 were measured with an ELISA. Seropositivity and natural infection levels were defined as described previously.[8] Seropositivity was defined as a titre greater than or equal to the assay threshold: 8 EU/mL for HPV16 and 7 EU/mL for HPV18.

A safety subset of women from selected study sites (Australia, Brazil, Canada, Finland, Mexico, Philippines, Thailand, and the USA) completed and returned safety diary cards documenting symptoms experienced during the first 7 days after vaccination (with a three-grade scale of symptom intensity) and symptoms within the first 30 days after vaccination. In all women during the entire study, reports of serious adverse events, new onset chronic disease including new onset autoimmune disease, medically significant conditions (adverse events prompting either

| | Vaccine group | Control group | Total |
|---|---|---|---|
| CIN2+ with HPV16 or HPV18 DNA in lesion | 2 | 21 | 23* |
| CIN2 with HPV16 or HPV18 DNA in lesion | 1 | 16 | 17 |
| CIN3 with HPV16 or HPV18 DNA in lesion | 1 | 5 | 6 |
| CIN2+ with HPV16 or HPV18 plus other oncogenic types of HPV | 2 | 12 | 14 |
| CIN2+ with several oncogenic types of HPV in which HPV16 or HPV18 detected for the first time | 2† | 1‡ | 3 |
| CIN2+ with HPV16 or HPV18 DNA in lesion and in preceding cytology sample | 0 | 20 | 20 |

*Seven cases with low-grade cytology (atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesions), 11 had HPV infection at entry. 14 cases had infection detected before month 6. †One case of CIN2, one case of CIN3. HPV18 and HPV58 DNA detected in the CIN2 lesion; patient had preceding infection with HPV58 at month 0 and 12. HPV16 and HPV58 DNA detected in the CIN3 lesion; patient had low-grade squamous intraepithelial lesion at month 0 and preceding infection with HPV58 at months 0, 6, and 12. Neither case showed E4 expression for the vaccine type being assessed. Only the punch biopsy of the CIN3 lesion showed HPV16 and HPV58; both CIN3 blocks from an excision specimen contained HPV58 only. ‡One case of CIN3 analysed for efficacy against HPV18 DNA was positive for both HPV16 and HPV18. This case was excluded from the analysis for HPV16 efficacy because of preceding infection with HPV16 detected at months 0, 6, and 12. Atypical squamous cells of undetermined significance (Hybrid Capture 2 positive) seen at month 0. Of the 10 areas of CIN3 examined, only one contained HPV18; all 10 areas contained HPV16.

*Table 3:* CIN2+ lesions with HPV16 or HPV18 DNA

| | Group | N | n | Woman-years at risk | Event rate* | Vaccine efficacy† | p |
|---|---|---|---|---|---|---|---|
| **CIN2+** | | | | | | | |
| Based on HPV16 or HPV18 DNA in the lesion (prespecified) | | | | | | | |
| Type 16/18 | Vaccine | 7788 | 2 | 9613·75 | 0·02 (0·00–0·09) | 90·4% (53·4 to 99·3) | <0·0001 |
| | Control | 7838 | 21 | 9682·00 | 0·22 (0·12–0·35) | | |
| Type 16 | Vaccine | 6701 | 1 | 8279·75 | 0·01 (0·00–0·08) | 93·3% (47·0 to 99·9) | 0·0005 |
| | Control | 6717 | 15 | 8284·32 | 0·18 (0·09–0·32) | | |
| Type 18 | Vaccine | 7221 | 1 | 8903·55 | 0·01 (0·00–0·07) | 83·3% (−78·8 to 99·9) | 0·1249 |
| | Control | 7258 | 6 | 8947·82 | 0·07 (0·02–0·16) | | |
| Based on HPV16 or HPV18 DNA in the lesion and in preceding cytology samples (additional analysis) | | | | | | | |
| Type 16/18 | Vaccine | 7788 | 0 | 9614·95 | 0·00 (0·00–0·05) | 100·0% (74·2 to 100·0) | <0·0001 |
| | Control | 7838 | 20 | 9682·45 | 0·21 (0·12–0·34) | | |
| Type 16 | Vaccine | 6701 | 0 | 8280·64 | 0·00 (0·00–0·06) | 100·0% (64·5 to 100·0) | <0·0001 |
| | Control | 6717 | 15 | 8284·32 | 0·18 (0·09–0·32) | | |
| Type 18 | Vaccine | 7221 | 0 | 8903·86 | 0·00 (0·00–0·05) | 100·0% (−49·5 to 100·0) | 0·0625 |
| | Control | 7258 | 5 | 8948·26 | 0·06 (0·01–0·15) | | |
| **CIN1+** | | | | | | | |
| Based on HPV16 or HPV18 DNA in the lesion (prespecified) | | | | | | | |
| Type 16/18 | Vaccine | 7788 | 3 | 9613·42 | 0·03 (0·00–0·10) | 89·2 (59·4 to 98·5) | <0·0001 |
| | Control | 7838 | 28 | 9681·19 | 0·29 (0·18–0·44) | | |
| Type 16 | Vaccine | 6701 | 2 | 8279·42 | 0·02 (0·00–0·10) | 88·9 (44·6 to 99·2) | 0·0004 |
| | Control | 6717 | 18 | 8283·71 | 0·22 (0·12–0·37) | | |
| Type 18 | Vaccine | 7221 | 1 | 8903·55 | 0·01 (0·00–0·07) | 90·9 (22·1 to 99·9) | 0·0063 |
| | Control | 7258 | 11 | 8947·48 | 0·12 (0·05–0·24) | | |
| Based on HPV16 or HPV18 DNA in the lesion and in preceding cytology samples (additional analysis) | | | | | | | |
| Type 16/18 | Vaccine | 7788 | 1 | 9614·62 | 0·01 (0·00–0·07) | 96·1 (71·6 to 100·0) | <0·0001 |
| | Control | 7838 | 26 | 9681·63 | 0·27 (0·16–0·42) | | |
| Type 16 | Vaccine | 6701 | 1 | 8280·31 | 0·01 (0·00–0·08) | 94·1 (54·3 to 99·9) | <0·0001 |
| | Control | 6717 | 17 | 8283·71 | 0·21 (0·11–0·35) | | |
| Type 18 | Vaccine | 7221 | 0 | 8903·86 | 0·00 (0·00–0·05) | 100·0 (33·8 to 100·0) | 0·0039 |
| | Control | 7258 | 9 | 8948·06 | 0·10 (0·04–0·21) | | |

Women assessed were DNA negative and seronegative for the corresponding HPV type at month 0. For combined HPV types women included in the analysis were infected with at least one oncogenic HPV type. *Data are cases per 100 woman-years (97·9% CI). †Data are % (97·9% CI).

*Table 4:* Efficacy against CIN2+ and CIN1+ associated with HPV16 or HPV18 in the total vaccinated cohort for efficacy

| | Group | N | n | Vaccine efficacy* | p |
|---|---|---|---|---|---|
| **6-month persistent infection with HPV16/18** | | | | | |
| DNA negative and seronegative at study entry | | | | | |
| Type 16/18 | Vaccine | 6344 | 38 | 80·4% (70·4 to 87·4) | <0·0001 |
| | Control | 6402 | 193 | | |
| Type 16 | Vaccine | 5493 | 23 | 84·1% (73·5 to 91·1) | <0·0001 |
| | Control | 5520 | 144 | | |
| Type 18 | Vaccine | 5896 | 15 | 74·0% (49·1 to 8·8) | <0·0001 |
| | Control | 5939 | 58 | | |
| **6-month persistent infection with oncogenic HPV types** | | | | | |
| Type-specific DNA negative at study entry | | | | | |
| Type 45 | Vaccine | 6724 | 10 | 59·9% (2·6 to 85·2) | 0·0165 |
| | Control | 6747 | 25 | | |
| Type 31 | Vaccine | 6615 | 47 | 36·1% (0·5 to 59·5) | 0·0173 |
| | Control | 6667 | 74 | | |
| Type 33 | Vaccine | 6702 | 31 | 36·5% (–9·9 to 64·0) | 0·0560 |
| | Control | 6736 | 49 | | |
| Type 52 | Vaccine | 6532 | 79 | 31·6% (3·5 to 51·9) | 0·0093 |
| | Control | 6573 | 116 | | |
| Type 58 | Vaccine | 6688 | 43 | –31·4% (–132·1 to 24·7) | 0·2515 |
| | Control | 6734 | 33 | | |
| Oncogenic HPV other than vaccine types† | Vaccine | 6773 | 505 | 9·0% (–5·1 to 21·2) | 0·1410 |
| | Control | 6804 | 554 | | |
| Oncogenic HPV‡ | Vaccine | 6773 | 545 | 21·9% (10·7 to 31·7) | <0·0001 |
| | Control | 6804 | 691 | | |
| **12-month persistent infection with HPV16/18** | | | | | |
| DNA negative and seronegative at study entry | | | | | |
| Type 16/18 | Vaccine | 3386 | 11 | 75·9% (47·7 to 90·2) | <0·0001 |
| | Control | 3437 | 46 | | |
| Type 16 | Vaccine | 2945 | 7 | 79·9% (48·3 to 93·8) | <0·0001 |
| | Control | 2972 | 35 | | |
| Type 18 | Vaccine | 3143 | 4 | 66·2% (–32·6 to 94·0) | 0·0766 |
| | Control | 3190 | 12 | | |
| **12-month persistent infection with oncogenic HPV types** | | | | | |
| Type-specific DNA negative at study entry | | | | | |
| Type 45 | Vaccine | 3584 | 3 | 62·3% (–93·2 to 95·4) | 0·2262 |
| | Control | 3601 | 8 | | |
| Type 31 | Vaccine | 3527 | 15 | 10·8% (–115·2 to 63·6) | 0·8598 |
| | Control | 3568 | 17 | | |
| Type 33 | Vaccine | 3574 | 6 | 45·1% (–91·8 to 86·5) | 0·3318 |
| | Control | 3603 | 11 | | |
| Type 52 | Vaccine | 3489 | 16 | 46·5% (–12·3 to 75·8) | 0·0533 |
| | Control | 3508 | 30 | | |
| Type 58 | Vaccine | 3563 | 6 | –1·1% (–372·0 to 78·4) | 1·000 |
| | Control | 3601 | 6 | | |
| Oncogenic HPV other than vaccine types† | Vaccine | 3611 | 100 | 27·1% (0·5 to 46·8) | 0·0174 |
| | Control | 3632 | 137 | | |
| Oncogenic HPV‡ | Vaccine | 3611 | 112 | 38·2% (18·0 to 53·7) | <0·0001 |
| | Control | 3632 | 180 | | |

Women have at least 5 months of follow-up for 6-month definition and at least 10 months of follow-up for 12-month definition. A woman with a 12-month persistent infection was also counted in the analysis for 6-month persistent infection. *Data are % (97·9% CI). †HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. ‡HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Combined oncogenic HPV types: women included in the analysis were infected with at least one oncogenic HPV type.

*Table 5:* **Vaccine efficacy against persistent infections with oncogenic HPV types in the total vaccinated cohort for efficacy**

## Statistical analysis

Interim assessment of the data was triggered with an event-defined analysis plan when at least 23 cases of CIN2+ associated with HPV16/18 DNA in the lesion were detected in the total vaccinated cohort for efficacy. This analysis assesses prophylactic efficacy in women uninfected with the individual HPV type at study entry (day 0). The total vaccinated cohort for efficacy included women who had prevalent infection with oncogenic HPV, often with several types, as well as low-grade cytological abnormalities at study entry and who received at least one vaccine dose. Efficacy analyses were thus done on a prespecified modified intention-to-treat basis. This analysis was selected to provide a conservative estimate of prophylactic vaccine efficacy in women uninfected with the vaccine type considered. All endpoints will be assessed in the fully vaccinated cohort (according-to-protocol cohort) in the final analysis.

The target enrolment of 18 000 unscreened women would provide 17 100 women negative at entry for HPV16 or HPV18 DNA (by PCR). At interim analysis, assuming a study dropout rate of 15% by month 12, we estimated that 14 534 women would be available to assess the primary endpoint. Assuming a CIN2+ yearly event rate of 0·27 cases per 100 women and vaccine efficacy against CIN2+ of 80%, the interim analysis would provide 83% power to confirm a 97·9% CI lower limit above 0%.[13] The overall alpha of 0·05 was split into 0·021 for the interim analysis and 0·039 for the final analysis.[14] No stopping rules were applied. To control the type I error, we sequentially assessed CIN2+ associated with HPV16/18, 6-month persistent infection with HPV16/18, CIN1+ associated with HPV16/18, 12-month persistent infection with HPV16/18, and 6-month persistent infection with 14 oncogenic types.

To maintain study blinding, an independent external statistician did all analyses with SAS version 8.2 and ProcStatXact 5.

Event rates were calculated as the number of cases divided by the accrued person-time since enrolment in both treatment groups and are expressed per 100 woman-years. CI around the event rates were determined by exact Poisson intervals around the number of cases and by division of the confidence limits by the accrued person-time.

Vaccine efficacy was defined as (1–rate ratio)×100%, which is the ratio of the event rates between the vaccinated and control groups. CI were calculated around the vaccine efficacy with a conditional exact method, which is used to compute an exact CI around the rate ratio.[15]

We defined statistical significance when the lower limit of the 97·9% CI was above 0. Additionally, p values were calculated with the Fisher's exact test to compare the proportion of events between vaccine and control groups. Case counting started the day after the first vaccination and ended at the time of an endpoint event

emergency room visits or physician visits that are not related to common diseases—eg, sinusitis and pharyngitis), and pregnancy and pregnancy outcomes were collected.

(eg, the time of the detection of CIN2+ or the start of persistent infection). If there was no event, the date of the last visit for which efficacy data was available was used.

Immunogenicity analyses were done per protocol in a subset of women that included those who met eligibility criteria, complied with protocol procedures, and had results for antibodies against at least one vaccine antigen. The size of the subset was determined to provide representative data to interpret the vaccine immune response. Seropositivity rates for HPV16 and HPV18 were calculated and geometric mean titres for each antigen are reported in ELISA units per mL (EU/mL) with 95% CI. Safety assessments were done in women with at least one vaccine dose administered (ie, the total vaccinated cohort).

This trial is registered with the US National Institutes of Health clinical trial registry, with the number NCT00122681.

### Role of funding source
The study sponsor designed the study and coordinated data collection. The results of this interim analysis have been submitted for regulatory purposes by the sponsor. JP, CMW, DLA, SRS, UJ, JS, WQ, FXB, BS, PP, FS, DJ, SLW, and GD had access to the complete interim study report. Because the trial is continuing, individual data could not be unblinded. The corresponding author had final responsibility for the decision to submit for publication.

### Results
The demographic characteristics of women enrolled in this study are shown in table 1. 17 106 (92%) women complied with the full three-dose vaccination schedule and only 5% dropped out from the study; reasons for dropouts are shown in table 1. The baseline characteristics for vaccinated women are shown in table 2. 3753 (20%) women had cervical oncogenic HPV DNA at baseline.

Almost all women vaccinated (99·4%) were included in the total vaccinated cohort for efficacy. The figure shows the number of women assessed and reasons for exclusion from each analysis. The mean follow-up time for women in the primary analysis for efficacy at the time of this analysis was about 14·8 (SD 4·9) months after the first vaccination.

The primary analysis in the total vaccinated cohort for efficacy (post-dose 1) occurred when 23 women had a CIN2+ lesion: two cases in the HPV16/18 vaccine group and 21 in the control group (table 3). Of these lesions, six were CIN3 and 17 were CIN2 containing HPV16/18 DNA. Based on the prespecified analysis, we estimated vaccine efficacy to be 90·4% (97·9% CI 53·4–99·3; p<0·0001; table 4). The vaccine also showed 89·2% efficacy against CIN1+ (59·4–98·5; p<0·0001).

14 of the 23 women had at least one other HPV type in the CIN2+ lesion (HPV31, 33, 35, 39, 51, 52, 56, 58,

or 68). Additional analyses showed that in three of these lesions, HPV16 or HPV18 was not found in any preceding cervical cytological sample, but a non-vaccine HPV type was detected in all histological sections of the CIN2+ and in preceding cytology samples, starting at month 0. Two of these cases also showed abnormal cytology at entry (table 3).

Exploratory molecular analysis of the cases of CIN2+ with several HPV types that were excluded from the additional analysis confirmed the presence of HPV DNA by type-specific PCR, except for the case in the vaccine

| | Vaccine group | Control group |
|---|---|---|
| **Solicited local adverse events within 7 days after vaccination in the safety subset*** | | |
| Number assessed | 3077 | 3080 |
| Pain | | |
| All | 2786 (90·5%) | 2402 (78·0%) |
| Grade 3 | 502 (16·3%) | 136 (4·4%) |
| Redness (mm) | | |
| All | 1348 (43·8%) | 851 (27·6%) |
| >50·0 | 37 (1·2%) | 3 (0·1%) |
| Swelling (mm) | | |
| All | 1292 (42·0%) | 609 (19·8%) |
| >50·0 | 74 (2·4%) | 15 (0·5%) |
| **Solicited general adverse events within 7 days after vaccination in the safety subset†** | | |
| Number assessed | 3076 | 3080 |
| Arthralgia | | |
| All | 633 (20·6%) | 551 (17·9%) |
| Grade 3 | 32 (1·0%) | 23 (0·7%) |
| Fatigue | | |
| All | 1771 (57·6%) | 1652 (53·6%) |
| Grade 3 | 126 (4·1%) | 99 (3·2%) |
| Fever‡ | | |
| All | 381 (12·4%) | 337 (10·9%) |
| >39·0°C | 18 (0·6%) | 10 (0·3%) |
| Gastrointestinal | | |
| All | 850 (27·6%) | 841 (27·3%) |
| Grade 3 | 60 (2·0%) | 61 (2·0%) |
| Headache | | |
| All | 1665 (54·1%) | 1579 (51·3%) |
| Grade 3 | 131 (4·3%) | 108 (3·5%) |
| Myalgia | | |
| All | 1606 (52·2%) | 1382 (44·9%) |
| Grade 3 | 141 (4·6%) | 47 (1·5%) |
| Rash | | |
| All | 312 (10·1%) | 258 (8·4%) |
| Grade 3 | 8 (0·3%) | 5 (0·2%) |
| Urticaria | | |
| All | 298 (9·7%) | 244 (7·9%) |
| Grade 3 | 29 (0·9%) | 30 (1·0%) |
| **Unsolicited events within 30 days of last vaccination in the safety subset** | | |
| Number assessed | 3184 | 3187 |
| Unsolicited symptom | 1354 (42·5%) | 1389 (43·6%) |

(Continues on next page)

| (Continued from previous page) | | |
|---|---|---|
| **Serious adverse events, new onset chronic disease, medically significant conditions during entire study in the total vaccinated cohort§** | | |
| Number assessed | 9319 | 9325 |
| Number of women reporting serious adverse event | 330 (3·5%) | 323 (3·5%) |
| Number of serious adverse events reported | 389 | 372 |
| Number of serious adverse events related to vaccination | 9 (0·1%) | 6 (0·1%) |
| Medically significant condition | 1988 (21·3%) | 2030 (21·8%) |
| New onset chronic disease¶ | 143 (1·5%) | 154 (1·7%) |
| New onset autoimmune disease¶ | 31 (0·3%) | 26 (0·3%) |

Data are n (%). *All solicited local symptoms were considered causally related to vaccination as per protocol. Grade 3 is any adverse event that prevents normal, everyday activities. †At the discretion of the investigator, myalgia could include both local and systemic muscle pain. ‡Fever was defined as axillary temperature 37·5°C/oral temperature 37·5°C. §All women in the total vaccinated cohort are included in this analysis. ¶Categories of new onset chronic disease and new onset autoimmune disease include (not necessarily occurring) immune system disorders: endocrine, musculoskeletal and connective tissue, metabolism and nutrition, respiratory and thoracic disorder.

*Table 6:* Safety outcomes

| | Vaccine group | Control group |
|---|---|---|
| Number of pregnancies | 665 | 685 |
| Number of ongoing pregnancies | 201 (30%) | 233 (34%) |
| Normal infant | 270 (41%) | 264 (39%) |
| Spontaneous abortion | 66 (10%) | 51 (7%) |
| Elective termination | 87 (13%) | 93 (14%) |
| Abnormal infant | 4 (1%) | 8 (1%) |

Some less frequent pregnancy outcomes not listed.

*Table 7:* Pregnancy and pregnancy outcomes in the total vaccinated cohort during the entire study

group with HPV18, for which the presence of HPV18 DNA was not confirmed. Laboratory contamination by HPV was carefully excluded and all cases were from different study sites. When we assessed E4 expression in all three cases, there was no HPV E4 gene expression associated with the HPV16 or HPV18 DNA detected in the lesion.

In women seronegative and DNA negative for HPV, a high level of protection was seen against persistent infections with HPV16 or HPV18: 80·4% (97·9% CI 70·4–87·4) against 6-month persistence and 75·9% (47·7–90·2) against 12-month persistence (table 5). 165 (71%) of the 6-month persistent infections and 53 (93%) of the 12-month persistent infections were acquired before the three-dose vaccination course was completed.

Table 4 also shows vaccine efficacy for the five most common HPV types in cervical cancer, after HPV16 and HPV18.[4,5] We observed cross-protection against 6-month persistent infections with HPV45 (vaccine efficacy 59·9%, 97·9% CI 2·6–85·2), HPV31 (36·1%, 0·5–59·5), and HPV52 (31·6%, 3·5–51·9) and broad protection against 12-month persistent infections with 12 combined oncogenic HPV types, not including HPV16 and HPV18 (27·1%, 0·5–46·8).

Over 99·5% of women (initially seronegative for corresponding vaccine type) seroconverted for both HPV16/18 post-dose 2 and post-dose 3 in the according-to-protocol immunogenicity subset (webfigure). Peak immune responses of 9341·5 (95% CI 8760·4–9961·1) EU/mL for HPV16 and 4769·6 (4491·2–5065·3) EU/mL for HPV18 were seen after dose 3 (month 7; webfigure). Geometric mean titres for natural infection antibody levels were 29·8 (28·5–31·0) EU/mL in women who had cleared HPV16 infection and 22·6 (21·6–23·6) EU/mL for HPV18.

Injection site symptoms (pain, redness, and swelling) were reported more frequently in the vaccine group than in the control group (table 6). However, most local symptoms were transient, with the mean duration of local

symptoms ranging from 2·2 to 3·4 days in both groups. The occurrence of solicited general symptoms within 7 days of vaccination was slightly higher in the vaccine group than in the control group. Fatigue, headache, and myalgia were reported more frequently in the vaccine group than in the control group (table 6). No increase in the occurrence of solicited local and general symptoms was seen with each subsequent dose. The proportion of women reporting new onset chronic disease, new onset autoimmune disease, and medically significant conditions was much the same in both groups (table 6).

In both groups, the most frequently reported serious adverse events were related to infectious events (data not shown) and abnormal pregnancy outcomes; there was no overall difference in pregnancy outcomes between the HPV vaccine and control groups (table 7). Five deaths (one in the vaccine group and four in the control group) were reported; however, these were deemed to be unrelated to vaccination.

## Discussion

This interim analysis shows that the adjuvanted HPV16/18 vaccine exhibits high prophylactic efficacy against CIN2+ associated with HPV16/18 in women who were uninfected with HPV16 or HPV18 but possibly infected with other oncogenic HPV types or who had current or previous low-grade cytological abnormalities before vaccination. No clinically meaningful differences were noted in safety outcomes between the study groups, although injection site symptoms and some solicited general symptoms were more common in the HPV vaccine group than they were in the control group. Furthermore, similar to previous immunogenicity studies with the same vaccine,[7,8] we saw a strong vaccine-induced antibody response for both HPV16 and HPV18. Thus our results show that the vaccine is effective, well tolerated, and immunogenic in a broad population of young adult women, lending support to its potential value in preventing CIN and cervical cancer.

The high proportion of lesions with several HPV types required an additional analysis to assign probable causality to cervical lesions. Persistence of type-specific oncogenic HPV infection has been consistently

associated with increased risk of CIN2+.[16–19] Taking into account preceding oncogenic HPV infection, we concluded that in the three cases with DNA from several HPV types, the HPV16 or HPV18 DNA that was detected in the lesion only were unlikely to be the cause of the lesion. The lesions were probably causally attributed to infection with different oncogenic types of HPV (HPV58 in two cases and HPV16 in one case) that were consistently detected from study entry before the diagnosis of CIN2+. As shown in the additional analysis, if these cases were excluded, the vaccine would show an estimated 100% (97·9% CI 74·2–100) prophylactic efficacy against CIN2+ attributed to HPV16 or HPV18.

Although the exploratory E4 immunostaining analysis was not used in the assessment of vaccine efficacy against CIN2+, it provided insights into the functional activity of HPV detected as DNA. The presence of HPV DNA in a CIN2+ lesion that was not associated with preceding infection or with E4 expression might represent contamination, transient carriage linked to sexual exposure, an early infection, or a latent HPV infection acquired before study entry that was undetected by cervical cytology samples but detected with biopsy. We plan to assess the biomolecular activity of HPV DNA detected in CIN2+ further, including type-specific HPV early gene expression.

This interim analysis was limited by the short follow-up and the small number of cases for some secondary and exploratory endpoints. A larger number of cases should be accrued by the time of final analysis, which will be triggered by cases of CIN2+ associated with HPV16/18 in the per-protocol population. Furthermore, longer follow-up should contribute to a better understanding of the biological importance and causal role of individual HPV types when more than one oncogenic HPV type is detected in a CIN2+ lesion, in addition to any clinical implication related to these outcomes. On the basis of current knowledge of established persistent infection with oncogenic HPV types and development of CIN2+, the persisting HPV type is probably the primary causal HPV type when several types are detected in a lesion. A consequence of the assessment of efficacy over a mean period of 15 months from start of vaccination was that a high proportion of the persistent HPV infections or incident CIN resulted from infection starting before month 6; as a result, our estimate of vaccine efficacy is probably conservative.

Data from this study extend the previously described cross-protection against incident infection with HPV45 and HPV31 to the more biologically relevant endpoint of persistent infection.[8] There is evidence that highly homologous HPV types—eg, HPV types 18 and 45 and HPV types 16 and 31—share cross-neutralising epitopes that could explain the partial efficacy seen previously.[20–22] Such protection against HPV45 and HPV31 would be valuable in view of the important role of these HPV types in causing an estimated 10% of cases of cervical cancer worldwide. We also noted vaccine efficacy against HPV52, but a previous study has suggested that such efficacy might not be sustained over time.[8] Additionally, we found broad protection against 12-month persistent infection with combined oncogenic, non-vaccine HPV types, suggesting that cross-protection might extend beyond types 45 and 31. However, the quantitative estimate of the effect of vaccine on individual non-vaccine HPV types is also limited by the short follow-up time, the possibility of infection starting before month 6, and the small numbers of cases. We anticipate additional cross-protection data at final analysis.

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