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Efficacy of the bivalent HPV vaccine against HPV 16/18-associated precancer: long-term follow-up results from the Costa Rica Vaccine Trial

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Summary

Background Oncogenic human papillomavirus (HPV) infections cause most cases of cervical cancer. Here, we report long-term follow-up results for the Costa Rica Vaccine Trial (publicly funded and initiated before licensure of the HPV vaccines), with the aim of assessing the efficacy of the bivalent HPV vaccine for preventing HPV 16/18-associated cervical intraepithelial neoplasia grade 2 or worse (CIN2+).

Methods Women aged 18–25 years were enrolled in a randomised, double-blind, controlled trial in Costa Rica, between June 28, 2004, and Dec 21, 2005, designed to assess the efficacy of a bivalent vaccine for the prevention of infection with HPV 16/18 and associated precancerous lesions at the cervix. Participants were randomly assigned (1:1) to receive an HPV 16/18 AS04-adjuvanted vaccine or control hepatitis A vaccine. Vaccines were administered intramuscularly in three 0·5 mL doses at 0, 1, and 6 months and participants were followed up annually for 4 years. After the blinded phase, women in the HPV vaccine group were invited to enrol in the long-term follow-up study, which extended follow-up for 7 additional years. The control group received HPV vaccine and was replaced with a new unvaccinated control group. Women were followed up every 2 years until year 11. Investigators and patients were aware of treatment allocation for the follow-up phase. At each visit, clinicians collected cervical cells from sexually active women for cytology and HPV testing. Women with abnormal cytology were referred to colposcopy, biopsy, and treatment as needed. Women with negative results at the last screening visit (year 11) exited the long-term follow-up study. The analytical cohort for vaccine efficacy included women who were HPV 16/18 DNA-negative at vaccination. The primary outcome of this analysis was defined as histopathologically confirmed CIN2+ or cervical intraepithelial neoplasia grade 3 or worse associated with HPV 16/18 cervical infection detected at colposcopy referral. We calculated vaccine efficacy by year and cumulatively. This long-term follow-up study is registered with ClinicalTrials.gov, NCT00867464.

Findings 7466 women were enrolled in the Costa Rica Vaccine Trial; 3727 received the HPV vaccine and 3739 received the control vaccine. Between March 30, 2009, and July 5, 2012, 2635 women in the HPV vaccine group and 2836 women in the new unvaccinated control group were enrolled in the long-term follow-up study. 2635 women in the HPV vaccine group and 2677 women in the control group were included in the analysis cohort for years 0–4, and 2073 women from the HPV vaccine group and 2530 women from the new unvaccinated control group were included in the analysis cohort for years 7–11. Median follow-up time for the HPV group was 11·1 years (IQR 9·1–11·7), 4·6 years (4·3–5·3) for the original control group, and 6·2 years (5·5–6·9) for the new unvaccinated control group. At year 11, vaccine efficacy against incident HPV 16/18-associated CIN2+ was 100% (95% CI 89·2–100·0); 34 (1·5%) of 2233 unvaccinated women had a CIN2+ outcome compared with none of 1913 women in the HPV group. Cumulative vaccine efficacy against HPV 16/18-associated CIN2+ over the 11-year period was 97·4% (95% CI 88·0–99·6). Similar protection was observed against HPV 16/18-associated CIN3—specifically at year 11, vaccine efficacy was 100% (95% CI 78·8–100·0) and cumulative vaccine efficacy was 94·9% (73·7–99·4). During the long-term follow-up, no serious adverse events occurred that were deemed related to the HPV vaccine. The most common grade 3 or worse serious adverse events were pregnancy, puerperium, and perinatal conditions (in 255 [10%] of 2530 women in the unvaccinated control group and 201 [10%] of 2073 women in the HPV vaccine group). Four women in the unvaccinated control group and three in the HPV vaccine group died; no deaths were deemed to be related to the HPV vaccine.

Interpretation The bivalent HPV vaccine has high efficacy against HPV 16/18-associated precancer for more than a decade after initial vaccination, supporting the notion that invasive cervical cancer is preventable.

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See **Online** for appendix

Research in context

Evidence before this study

Large prelicensure clinical trials for the bivalent and quadrivalent human papillomavirus (HPV) vaccines have shown that both vaccines provide high vaccine efficacy against persistent infection with HPV 16 and 18 and associated cervical intraepithelial neoplasia grade 2 or worse (CIN2+) in women with no evidence of infection at vaccination. We searched PubMed from inception to Dec 20, 2019, for studies published in English of the long-term efficacy of the HPV vaccines against cervical precancer. We included any publications containing the following search terms in the title or abstract: "(HPV AND vaccine); (HPV AND vaccine AND bivalent); (HPV AND vaccine AND quadrivalent); (HPV AND vaccine AND nonavalent)". The longest reported duration of active follow-up for cervical precancer was 6 years for the bivalent vaccine, 3 years for the quadrivalent vaccine, and 6 years for the nonavalent vaccine.

Added value of this study

We report the efficacy of the bivalent vaccine to prevent cervical precancer (cervical intraepithelial neoplasia grade 2 or cervical intraepithelial neoplasia grade 3) associated with HPV 16/18 cervical infection, 11 years after initial vaccination in the Costa Rica HPV Vaccine Trial. We found that women vaccinated with the bivalent vaccine had protection against cervical intraepithelial neoplasia grade 3 or worse (CIN3+), the immediate precursor of invasive cervical cancer. To our knowledge, this is the longest follow-up of the protection provided by the bivalent vaccine against cervical precancer associated with HPV 16/18 infection.

Implications of all the available evidence

This long-term follow-up analysis of the Costa Rica Vaccine Trial demonstrates prolonged protection by the bivalent HPV vaccine against CIN2+ and CIN3+ caused by HPV 16 and 18 in women who were HPV 16/18 DNA-negative at initial vaccination. Between years 7 and 11 of follow-up, no women developed CIN2+ or CIN3+ in the HPV-vaccinated group despite continued disease detection in the unvaccinated control group. This finding suggests that the HPV vaccine results in prolonged protection against clinical disease, thus supporting the notion that invasive cervical cancer is preventable.

Introduction

Persistent infection with specific types of human papillomavirus (HPV) causes most cervical cancers.[1] Annually, 570 000 new cases of cervical cancer occur worldwide, of which 70% are attributable to HPV 16 and 18.[2] Mortality remains high in low-resource countries and lower socioeconomic groups.

Safe and effective vaccines against HPV have been available since 2006, and WHO recommends vaccination of adolescent girls in all countries.[3] Three vaccines have been prequalified by WHO: a bivalent vaccine against HPV 16 and 18; a quadrivalent vaccine against HPV 6, 11, 16, and 18; and a nonavalent vaccine against HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58.

In large prelicensure trials, bivalent and quadrivalent vaccines had high efficacy against HPV 16 and 18 persistent infection and associated cervical intraepithelial neoplasia grade 2 or worse (CIN2+) in women without infection at vaccination (vaccine efficacy >90%).[4–6] Nonavalent vaccines resulted in non-inferior antibody responses against HPV 6, 11, 16, and 18 when compared with quadrivalent vaccines, and 96·7% efficacy (95% CI 80·9–99·8) against HPV 31, 33, 45, 52, and 58-related high-grade lesions.[7] However, few studies have assessed the long-term efficacy of these vaccines against cervical precancer (ie, cervical intraepithelial neoplasia grade 2 [CIN2] or cervical intraepithelial neoplasia grade 3 [CIN3]). In clinical trials, the longest follow-up was 6 years for the bivalent vaccine,[8,9] 3 years for the quadrivalent vaccine,[4] and 6 years for the nonavalent vaccine.[10]

Consolidation of data on protection against advanced cancer precursors and assessment of long-term efficacy is crucial, since durable prophylactic HPV vaccine protection is necessary for lifelong reduction of cervical cancer risk.[11]

Here, we present long-term follow-up results for the Costa Rica Vaccine Trial (ClinicalTrials.gov, NCT00128661). The Costa Rica Vaccine Trial was publicly funded and initiated before HPV vaccine licensure. We aimed to assess the efficacy of the vaccine for preventing CIN2+ and CIN grade 3 or worse (CIN3+) associated with incident cevical infection with HPV 16, HPV 18, or both (referred to as HPV 16/18 hereafter), 11 years after vaccination.

Methods

Study design and participants

Women included in this study were participants in the double-blind, randomised Costa Rica Vaccine Trial, designed to assess the efficacy of a bivalent vaccine for the prevention of infection with HPV 16/18 and associated precancerous lesions at the cervix. Study design details have been published previously.[12] Briefly, women who resided in the Guanacaste and Puntarenas provinces of Costa Rica were enrolled between June 28, 2004, and Dec 21, 2005. Eligible women were aged 18–25 years, who planned to reside in Guanacaste province and surrounding areas for 6 months after first vaccination, understood Spanish, were generally in good health, and were willing to provide written informed consent. The trial was approved by the Institutional Review Boards of Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA) in Costa Rica and the National Cancer Institute (Bethesda, MD, USA) in the USA, and all women provided written informed consent.

At the year 4 follow-up visit of the Costa Rica Vaccine Trial, women in the HPV vaccine group were invited to enrol in the long-term follow-up study, which extended follow-up for 7 additional years. Detailed methods of extended follow-up have been reported previously.[13] Women from the control group of the Costa Rica Vaccine Trial were offered the bivalent HPV vaccine at the end of the 4-year blinded phase and attended one final follow-up visit 2 years after vaccination, after which they were exited from the long-term follow-up phase. Women who agreed to participate in the long-term follow-up study signed new written, informed consent forms.

Since HPV vaccination was offered to the control group after the 4-year follow-up visit (71% received at least one dose), a new screening-only, unvaccinated control group was recruited into the long-term follow-up study to replace the original control group. Enrolment in the unvaccinated control group occurred contemporaneously with participants of the Costa Rica Vaccine Trial who attended the year 4 visit and included women from the same birth cohorts in the same geographical regions as the original participants. The unvaccinated control group were not randomly assigned; thus, the long-term follow-up study is considered an epidemiological cohort study, rather than a randomised clinical trial.

Randomisation and masking

Women were randomly assigned (1:1) to receive either the AS04-adjuvanted HPV 16/18 vaccine (Cervarix; GlaxoSmithKline Biologicals, Rixensart, Belgium) or a control hepatitis A vaccine (Havrix; GlaxoSmithKline Biologicals). Randomisation was done using a blocked randomisation procedure with permuted block sizes of 14, 16, and 18.

Both vaccines were assigned vaccine identification numbers by staff at the National Cancer Institute using SAS (version 8.2). Labels containing the randomised numbers were provided to the vaccine manufacturer. Labelled syringes were combined, numerically ordered, and delivered in sequentially numbered boxes to the study site in Costa Rica. At the study clinics, the clinical staff pulled syringes in numerical order and applied the first dose of the vaccine. Participants, study personnel, and investigators were masked to treatment group assignment. Masking was maintained throughout the 4-year blinded phase of the Costa Rica Vaccine Trial. After this period, participants were informed about their vaccine status and were offered the study vaccine if they did not receive the HPV vaccine at enrolment. Thus, there was no masking in the long-term follow-up study.

Procedures

At the enrolment visit, pelvic examinations were done in women who were sexually active to collect cervical cells using a Cervex-Brush rinsed in PreservCyt solution (Hologic, Marlborough, MA, USA), for cytological assessment and HPV DNA testing.

Women were randomly assigned to receive either the HPV vaccine or a control hepatitis A vaccine. Participants were vaccinated intramuscularly in the deltoid muscle and received three 0·5 mL doses at 0, 1, and 6 months. Since not all women had pelvic examinations at the 6-month visit, all women provided a self-collected cervicovaginal sample for HPV testing at the 6-month visit.[14]

Pelvic examinations were done at annual follow-up visits, to obtain exfoliated cervical cells for cytological assessment and HPV DNA testing.

Women were divided into analytical cohorts on the basis of HPV status at enrolment and the 6-month visit. Colposcopy referral was based on cytology with HPV triage of atypical squamous cells of undetermined significance (ASC-US). Women with low-grade squamous intraepithelial lesions, HPV-positive ASC-US, or inadequate cytology at any visit were followed up every 6 months. Women with high-grade squamous intraepithelial lesion (HSIL) or with persistent minor abnormalities were referred to colposcopy. After colposcopy or treatment, screening continued every 6 months. Women returned to yearly follow-up after three consecutive normal cytology results or were referred to colposcopy again if they had HPV-positive ASC-US or worse.

At the end of the 4-year blinded phase in the Costa Rica Vaccine Trial, to assure safety of participants with regard to cervical disease risk, colposcopy referral criteria were modified to include a history of more than 2 years of persistent HPV 16/18 infection. Women with incident HPV 16/18 infection or persistent infection with oncogenic HPV other than HPV 16/18, and those with low-grade squamous intraepithelial lesions, HPV-positive ASC-US, or inadequate cytology at year 4 continued screening every 6 months.

In the long-term follow-up study, women in the unvaccinated control group had cervical screening at enrolment followed by an aggressive colposcopy referral algorithm to identify and treat prevalent disease, to increase their comparability with women included in the Costa Rica Vaccine Trial who had received annual screening for the previous 4 years.

For the long-term follow-up study, both the HPV vaccine group and the unvaccinated control group, had cytological screening every 2 years. Women with low-grade squamous intraepithelial lesions, HPV-positive ASC-US, or inadequate cytology had accelerated screening at 6 months with cytology and a HPV test. If both tests were normal, women returned to screening every 2 years. If the cytology was abnormal, women were referred to colposcopy. If the cytology was normal and the HPV test was positive, they had a second accelerated screening at 6 months; if either test was positive, they were referred to colposcopy. Women with HSIL were referred to colposcopy.

At the final screening visit of the long-term follow-up study (year 11), participants had cytological screening and HPV testing and those with negative results were exited from the study. Women with abnormal results and

participants in the accelerated follow-up who did not attend the last screening visit were invited to another screening visit or referred to colposcopy before exit.

For safety analyses during the long-term follow-up study, we documented serious adverse events independent of their possible association with vaccination, and pregnancy outcome data were collected and followed until resolution, as previously described.[13] Safety data from the Costa Rica Vaccine Trial have been reported previously.[5] Clinically significant conditions were defined as grade 3 (severe) events, events with life-threatening consequences were defined as grade 4, and deaths were defined as grade 5 events.

Cytology was reported using the Bethesda system.[15] Clinical management was based on cytology assessed in Costa Rica. For quality control, during the blinded phase of the Costa Rica Vaccine Trial, slides interpreted as abnormal in Costa Rica and a 10% random sample of negatives were re-read by one cytotechnologist and one pathologist from the USA. At the year 4 visit, slides interpreted with reactive changes from women identified as HPV-positive by the Hybrid Capture 2 test (Qiagen, Hilden, Germany) were also re-interpreted. If cytology was upgraded in the USA, this led to colposcopy referral. This quality control process was terminated in 2011 because only 0·56% of slides upgraded by the reviewers had histologically confirmed CIN2+.

Histological slides from biopsies or loop electrosurgical excisional procedure (LEEP) specimens were interpreted by a pathologist (DG) in Costa Rica for clinical management, and a blinded pathologist (TMD) in the USA reviewed all slides. Discrepant diagnoses were reviewed by a second pathologist (MHS) in the USA and a final diagnosis was assigned on the basis of majority rule. The presence of CIN2 was not confirmed by p16 immunostaining.

The Hybrid Capture 2 test was used for the detection of high-risk HPV types for clinical management and triage of women with ASC-US. At the year 11 visit, this test was replaced by the Aptima HPV assay (Hologic, San Diego, CA, USA). The performance of both tests has been shown to be similar.[16]

Cervical samples were tested for HPV DNA using the SPF10 PCR Primer System and a DNA enzyme immunoassay (DEIA) with the line-probe assay 25 assay (Labo Bio-medical Products, Rijswijk, Netherlands) at DDL Diagnostic Laboratory (Delft, Netherlands) during the blinded phase of the Costa Rica Vaccine Trial, and in later years the test was replaced by TypeSeq (National Cancer Institute Cancer Genomics Research Laboratory, Frederick, MD, USA) after careful evaluation and demonstration of their comparability. Overall and positive agreement was high and no difference in vaccine efficacy was observed when using either test to define outcomes.[17]

During the blinded phase of the Costa Rica Vaccine Trial, extracted DNA from cervical specimens was used for amplification with SPF10 primers followed by DEIA detection of amplimers, as described previously.[12] Extracted DNA from cervical specimens was used for amplification with SPF10 primers followed by DEIA detection of amplimers. The same amplimers were used on SPF10-DEIA-positive samples to identify genotype by reverse hybridisation with the line-probe assay 25. Specimens positive by SPF10-DEIA but negative for HPV 16 or HPV 18 by line-probe assay 25 were tested for HPV 16 and HPV 18 using type-specific primers.[18]

TypeSeq assays were done at the National Cancer Institute Cancer Genomics Research Laboratory using the TypeSeq 3-PCR stage workflow. HPV genotyping was done by Ion S5 next-generation sequencing followed by custom Torrent Suite plugin analysis (Thermo Fisher Scientific, Waltham, MA, USA). A binary result of positive or negative was reported for the human positive control and for each of the 51 HPV types detected by the assay.[19]

Outcomes

Three outcomes were prespecified for the long-term follow-up study: assessment of the long-term efficacy and safety of HPV 16/18 vaccination; assessment of determinants of the immune response to HPV and the vaccine; and the effect of the vaccine on the natural history of HPV and cervical disease. Here, we present the primary histological outcome, defined as a final diagnosis of CIN2+ or CIN3+ that was associated with HPV 16/18 cervical infection in the cervical cytology specimen that led to colposcopy referral, and serious adverse events reported during long-term follow-up. In our previous report of the blinded phase of the Costa Rica Vaccine Trial, an alternative definition for the attribution of HPV genotype associated with CIN2+ lesions was used, which did not affect vaccine efficacy. That definition considered evidence of HPV persistence preceding referral to colposcopy when attributing HPV types to lesions in instances when more than one HPV type was present in the cervical cytology specimen that led to colposcopy referral.[5] Efficacy against virological endpoints has been reported separately[20,21] and safety data from the blinded phase of Costa Rica Vaccine Trial have been reported previously.[5] Immune response correlates of protection endpoints are not reported here because of the low number of breakthrough infections. Analyses of the natural history of HPV and cervical cancer are ongoing, and will be reported elsewhere.

Statistical analysis

Sample size was calculated for the randomised blinded phase of the trial. For the epidemiological follow-up, we continued to follow up the majority of women in the HPV vaccinated group and aimed to enrol 3000 women in the unvaccinated control group to provide a sample size similar to the original control group of the Costa Rica Vaccine Trial.[13]

The analytical cohort for the HPV vaccine group for our vaccine efficacy analysis included all women who

received three doses of the HPV 16/18 vaccine within protocol-defined windows (21–90 days between doses 1 and 2; 90–210 days between doses 2 and 3), who were HPV 16/18 DNA-negative at months 0 and 6, who did not have biopsy or LEEP during the vaccination phase, without an investigational new drug safety report during the vaccination period, and who otherwise complied with the protocol during the vaccination period. The analytical cohort (years 0–4) for the control group included all women from the original control group of the Costa Rica Vaccine Trial who fulfilled the same criteria as that for the HPV vaccine group. The analytical cohort (years 7–11) for the unvaccinated control group included all women who did not have a LEEP during the strict colposcopy algorithm applied at enrolment.

For sensitivity analyses, we defined an inclusive cohort, which provided a worst-case scenario of vaccine efficacy by including vaccinated women regardless of baseline HPV infection. This cohort included women from the HPV vaccine group following the same criteria defined for the main analysis cohort, but did not exclude women who were HPV 16/18 DNA-positive at months 0 and 6. Any participants (vaccinated or unvaccinated) who had a LEEP during a previous visit were excluded from the inclusive cohort because after a LEEP procedure, women are no longer within the at-risk population because they are unlikely to develop CIN2+ in such a short period of time.

This analysis aimed to investigate durability of the vaccine efficacy against histological endpoints. We prespecified two analytical approaches: to assess the latest timepoints, to avoid higher early estimates driving overall efficacy, which could mask waning protection in later years of follow-up; and to assess cumulative efficacy to define the total benefit of HPV vaccination over time.

We divided the study period into eight non-overlapping periods. We defined time periods for each woman on the basis of time relative to enrolment dates (appendix p 5). For each period and vaccination group, we reported the number of women attending at least one examination visit, the number of women with a detectable CIN2+ or CIN3+, and the corresponding incidence (number of women with a detectable CIN2+ or CIN3+ divided by the number of women attending at least one examination visit). We then calculated the vaccine efficacy as 1 minus the incidence in the HPV vaccine group divided by the incidence in the control group. We calculated the exact CI for each incidence using a mid-p correction and the CI for each vaccine efficacy using a two-step approach.[21,22] For each period and cohort, we also reported cumulative incidence, using a Kaplan-Meier analysis and for each period we report the corresponding cumulative vaccine efficacy. Because of the small number of observed events, we calculated the CI for cumulative incidence using the beta product confidence procedure[23] and a conservative CI for cumulative vaccine efficacy by using the ratio of boundary points for the cumulative incidence CIs.

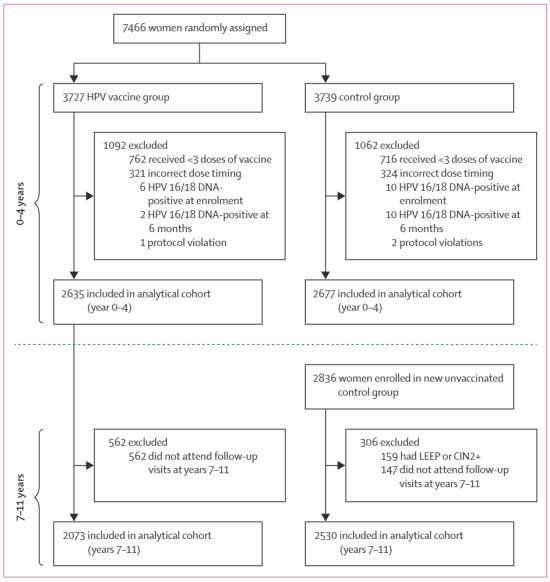

Figure: **Trial profile**
HPV=human papillomavirus. LEEP=loop electrosurgical excisional procedure. CIN2+=cervical intraepithelial neoplasia grade 2 or worse.

Women were censored and excluded for further analysis at diagnosis of CIN2+ or CIN3+. Additionally, women from the new unvaccinated control group were enrolled in the study at year 4, but were left-censored and thus did not contribute data for analysis between years 4 and 7.

To account for minor differences in the demographics of the HPV vaccine group and unvaccinated control group, we did a sensitivity analysis by calculating weighted estimates of incidence in the unvaccinated control group, with individuals inversely weighted by their propensity for being in the unvaccinated control group. Propensity scores were built using logistic regression with vaccination group as the dependent variable and age, lifetime sexual partners, marital status, and number of pregnancies as the independent variables. When defining cohorts, limiting the analytical cohort to only HPV-vaccinated women without a baseline infection could potentially bias results in favour of the vaccine. Therefore, we did a second sensitivity analysis, in which we repeated our primary analyses using an inclusive cohort, which excluded baseline HPV status. All statistical analyses were done using SAS (version 9.4). This study is registered with ClinicalTrials.gov, NCT00867464.

	Women, n	Women with CIN2+, n	Incidence per 100 women (95% CI)	Cumulative incidence per 100 women (95% CI)	Vaccine efficacy (95% CI)	Cumulative vaccine efficacy (95% CI)
Year 0						
HPV vaccine group	2635	0	0·00 (0·00 to 0·11)	0·00 (0·00 to 0·11)	NA	NA
Control group	2677	0	0·00 (0·00 to 0·11)	0·00 (0·00 to 0·11)	..	..
Year 1						
HPV vaccine group	2551	1	0·04 (0·00 to 0·19)	0·04 (0·00 to 0·19)	NC	NC
Control group	2586	0	0·00 (0·00 to 0·12)	0·00 (0·00 to 0·12)	..	..
Year 2						
HPV vaccine group	2488	0	0·00 (0·00 to 0·12)	0·04 (0·00 to 0·20)	100% (−184·7 to 100·0)	0·1% (−996·3 to 99·0)
Control group	2549	1	0·04 (0·00 to 0·19)	0·04 (0·00 to 0·19)	..	..
Year 3						
HPV vaccine group	2429	0	0·00 (0·00 to 0·12)	0·04 (0·00 to 0·20)	100% (−13·8 to 100·0)	80·5% (−168·2 to 99·6)
Control group	2479	4	0·16 (0·05 to 0·39)	0·20 (0·07 to 0·44)	..	..
Year 4						
HPV vaccine group	2477	1	0·04 (0·00 to 0·20)	0·08 (0·01 to 0·26)	94·0% (66·9 to 99·7)	90·9% (52·8 to 99·0)
Control group	2527	17	0·67 (0·41 to 1·05)	0·87 (0·56 to 1·30)	..	..
Year 7						
HPV vaccine group	1950	0	0·00 (0·00 to 0·15)	0·08 (0·01 to 0·28)	100% (18·6 to 100·0)	92·9% (62·5 to 99·2)
Unvaccinated new control group	2451	6	0·24 (0·10 to 0·51)	1·11 (0·76 to 1·59)	..	..
Year 9						
HPV vaccine group	1815	0	0·00 (0·00 to 0·16)	0·08 (0·01 to 0·29)	100% (57·0 to 100·0)	94·9% (74·0 to 99·4)
Unvaccinated new control group	2236	10	0·45 (0·23 to 0·80)	1·56 (1·12 to 2·11)	..	..
Year 11						
HPV vaccine group	1913	0	0·00 (0·00 to 0·16)	0·08 (0·01 to 0·29)	100% (89·2 to 100·0)	97·4% (88·0 to 99·6)
Unvaccinated new control group	2233	34	1·52 (1·07 to 2·10)	3·06 (2·42 to 3·82)	..	..

HPV=human papillomavirus. CIN2+=cervical intraepithelial neoplasia grade 2 or worse. NA=not applicable. NC=not calculable.

Table 1: **Vaccine efficacy against HPV 16/18-associated CIN2+ in the analytical cohort**

Role of the funding source

In collaboration with the Costa Rica Vaccine Trial investigators, the funder of the study had a role in the study design, data collection, data management, data analysis, data interpretation, and the writing of the report. GlaxoSmithKline Biologicals provided vaccine and support for aspects of the trial associated with regulatory submission needs of the company under a Clinical Trials Agreement (US Food and Drug Administration BB-IND 7920) during the randomised blinded phase of our study, but had no role in study design, data collection, data management, data analysis, data interpretation, or the writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between June 28, 2004, and Dec 21, 2005, 7466 women were enrolled in the Costa Rica Vaccine Trial (3727 in the HPV vaccine group; 3739 in the control group). Between March 30, 2009, and July 5, 2012, 2635 women in the HPV vaccine group and 2836 unvaccinated women (new control group) were enrolled in the long-term follow-up study. For the long-term follow-up phase, 2635 women in the HPV vaccine group and 2677 women in the control group were included in the analysis cohort for years 0–4, and 2073 women from the HPV vaccine group and 2530 women from the new unvaccinated control group were included in the analysis cohort for years 7–11 (figure).

Median follow-up time for the HPV vaccinated group was 11·1 years (IQR 9·1–11·7). For the unvaccinated groups, median follow-up time was 4·6 years (IQR 4·3–5·3) in the original control group and 6·2 years (5·5–6·9) in the unvaccinated new control group. Baseline characteristics of the vaccinated group and the original control group included in the cohort for efficacy were similar.[5] The women were similar with respect to age, area of residence, and number of lifetime sexual partners, but women in the unvaccinated control group were more likely to be married and had more pregnancies than the women in the original control group.[13] Comparisons between the original and new control groups showed that baseline characteristics and future risk for cervical HPV acquisition were similar between

	Women, n	Women with CIN3+, n	Incidence per 100 women (95% CI)	Cumulative incidence per 100 women (95% CI)	Vaccine efficacy (95% CI)	Cumulative vaccine efficacy (95% CI)
Year 0						
HPV vaccine group	2635	0	0·00 (0·00 to 0·11)	0·00 (0·00 to 0·11)	NA	NA
Control group	2677	0	0·00 (0·00 to 0·11)	0·00 (0·00 to 0·11)	..	..
Year 1						
HPV vaccine group	2551	1	0·04 (0·00 to 0·19)	0·04 (0·00 to 0·19)	NC	NC
Control group	2586	0	0·00 (0·00 to 0·12)	0·00 (0·00 to 0·12)	..	..
Year 2						
HPV vaccine group	2488	0	0·00 (0·00 to 0·12)	0·04 (0·00 to 0·20)	-	NC
Control group	2549	0	0·00 (0·00 to 0·12)	0·00 (0·00 to 0·12)	..	..
Year 3						
HPV vaccine group	2429	0	0·00 (0·00 to 0·12)	0·04 (0·00 to 0·20)	-	NC
Control group	2480	0	0·00 (0·00 to 0·12)	0·00 (0·00 to 0·12)	..	..
Year 4						
HPV vaccine group	2477	1	0·04 (0·00 to 0·20)	0·08 (0·01 to 0·26)	83·0% (−15·4 to 99·3)	66·4% (−175·4 to 97·3)
Control group	2532	6	0·24 (0·10 to 0·49)	0·24 (0·10 to 0·49)	..	..
Year 7						
HPV vaccine group	1950	0	0·00 (0·00 to 0·15)	0·08 (0·01 to 0·28)	100% (−40·1 to 100·0)	80·1% (−39·5 to 98·1)
Unvaccinated new control group	2451	4	0·16 (0·05 to 0·39)	0·40 (0·20 to 0·71)	..	..
Year 9						
HPV vaccine group	1815	0	0·00 (0·00 to 0·16)	0·08 (0·01 to 0·29)	100% (44·0 to 100·0)	89·5% (37·0 to 98·9)
Unvaccinated new control group	2238	8	0·36 (0·17 to 0·68)	0·76 (0·46 to 1·17)	..	..
Year 11						
HPV vaccine group	1913	0	0·00 (0·00 to 0·16)	0·08 (0·01 to 0·29)	100% (78·8 to 100·0)	94·9% (73·7 to 99·4)
Unvaccinated new control group	2237	18	0·80 (0·49 to 1·24)	1·56 (1·11 to 2·13)	..	..

HPV=human papillomavirus. CIN3+=cervical intraepithelial neoplasia grade 3 or worse. NA=not applicable. NC=not calculable.

Table 2: **Vaccine efficacy against HPV 16/18-associated CIN3+ in the analytical cohort**

the two groups.[13] Furthermore, vaccine efficacy estimates against one-time prevalent cervical HPV infection 4 years after vaccination using either the original control group or the unvaccinated control group were comparable.[13]

During 11 years of follow-up, in the efficacy analysis cohort, we observed an efficacy of 100% against incident HPV 16/18-associated CIN2+ in each year, with the exception of years 1 and 4 (table 1). Of the two cases of HPV 16/18-associated CIN2+ identified in the HPV vaccine group, the first woman developed CIN2+ in year 1, and was positive for antibodies against both HPV 16 and HPV 18 and had an HSIL cytology (upgraded from the cytology quality control process) at enrolment. She was positive for HPV 16 and HPV 45 at 11 months and diagnosed with CIN3 at 15 months after enrolment. The second woman had antibodies against both HPV 16 and HPV 18 at enrolment and was positive for HPV 16 DNA at 13 months after enrolment, remaining HPV 16-positive until the diagnosis of CIN3 at 78 months after enrolment.

At 11 years post-vaccination, the efficacy against incident HPV 16/18-associated CIN2+ was 100% (95% CI 89·2–100·0), and 34 (1·5%) of 2233 women in the unvaccinated group had developed CIN2+. Cumulative efficacy against CIN2+ was 97·4% (95% CI 88·0–99·6). Less than 1% of the patients with CIN2+ had cancer or adenocarcinoma in situ.

Vaccine efficacy against incident HPV 16/18-associated CIN3+ at 11 years post-vaccination was 100% (95% CI 78·8–100·0), and 18 (0·8%) of 2237 women in the unvaccinated control group had developed CIN3+. The cumulative efficacy against CIN3+ was 94·9% (95% CI 73·7–99·4; table 2).

We did several sensitivity analyses with adjustment for age, number of lifetime sexual partners, marital status, and number of pregnancies, to account for the comparisons in the long-term follow-up study (year 7 and later) since it was not randomised. We assessed protection against CIN2+ in the inclusive cohort. At year 11, the vaccine had high efficacy against both HPV 16/18-associated CIN2+ (93·5%, 95% CI 77·3–98·9) and CIN3+ (88·3%, 57·0–98·1; appendix pp 1–2). We also recalculated the incidence of HPV 16/18-associated CIN2+ using propensity score weighting to account for

	Unvaccinated new control group (n=2530)			HPV vaccine group (n=2073)		
	Grade 3	Grade 4	Grade 5	Grade 3	Grade 4	Grade 5
Infections and infestations	22 (<1%)	0	0	14 (<1%)	0	2 (<1%)
Autoimmune disorder	4 (<1%)	0	0	0	0	0
Blood and lymphatic system disorders	3 (<1%)	0	0	1 (<1%)	0	0
Cardiac disorders	2 (<1%)	0	1 (<1%)	1 (<1%)	0	1 (<1%)
Congenital, familial, and genetic disorders	0	0	0	1 (<1%)	0	0
Endocrine disorders	3 (<1%)	0	0	1 (<1%)	0	0
Gastrointestinal disorder	5 (<1%)	0	0	7 (<1%)	0	0
General disorders	1 (<1%)	0	0	1 (<1%)	0	0
Injury, poisoning, or procedural complications	6 (<1%)	1 (<1%)	1 (<1%)	7 (<1%)	1 (<1%)	0
Metabolism and nutrition disorders	2 (<1%)	0	0	1 (<1%)	0	0
Musculoskeletal and connective tissue disorders	4 (<1%)	0	0	0	0	0
Benign, malignant, and unspecified neoplasms	10 (<1%)	0	2 (<1%)	12 (<1%)	0	0
Nervous system disorders	3 (<1%)	0	0	3 (<1%)	0	0
Pregnancy, puerperium, and perinatal conditions	255 (10%)	0	0	201 (10%)	0	0
Psychiatric disorders	1 (<1%)	0	0	3 (<1%)	1 (<1%)	0
Renal and urinary disorders	4 (<1%)	0	0	3 (<1%)	0	0
Reproductive system and breast disorders	26 (1%)	0	0	15 (<1%)	0	0
Respiratory, thoracic, and mediastinal disorders	4 (<1%)	0	0	1 (<1%)	0	0
Skin and subcutaneous tissue disorders	1 (<1%)	0	0	2 (<1%)	0	0
Vascular disorders	0	0	0	1 (<1%)	0	0

Data are n (%). Each disease category includes the number of women with at least one grade 3 adverse event, thus, women could contribute to multiple disease categories. During the long-term follow-up, only serious adverse events (≥grade 3) were reported; <2% of participants had grade 1 (mild) or grade 2 (moderate) adverse events, and are therefore not shown here.

Table 3: **Serious adverse events reported during the long-term follow-up study in the analytical cohort**

the minor differences in demographic characteristics between the HPV vaccine group and unvaccinated control group (appendix pp 3–4). The adjusted incidence per 100 women was 0·29 (95% CI 0·09–0·66) at year 7, 0·38 (0·17–0·73) at year 9, and 1·50 (1·02–2·11) at year 11. For the CIN3+ outcome, the adjusted incidence per 100 women was 0·20 (95% CI 0·05–0·56) at year 7, 0·31 (0·13–0·65) at year 9, and 0·76 (0·44–1·23) at year 11, which similar to the unweighted incidence (tables 1 and 2).

During the long-term follow-up, no serious adverse events occurred that were deemed related to the HPV vaccine. Serious adverse events were similar in the unvaccinated control group and HPV vaccine group (table 3). The most common clinically significant grade 3 adverse events were pregnancy, puerperium, and perinatal conditions (255 [10%] of 2530 women in the unvaccinated control group; 201 [10%] of 2073 women in the HPV vaccine group). One grade 4 adverse event occurred in the unvaccinated control group (one injury, poisoning, or procedural complication) and two grade 4 adverse events were reported in the HPV group (one psychiatric disorder and one injury, poisoning, or procedural complication). Four women in the unvaccinated control group and three in the HPV vaccine group died; none of the deaths were deemed to be related to the HPV vaccine.

Discussion

This long-term follow-up analysis of the Costa Rica Vaccine Trial demonstrates that the bivalent HPV vaccine had almost 100% efficacy against the development of CIN2+ caused by HPV 16 and 18 among women who were HPV 16/18-negative at initial vaccination. The protection was also observed at the 11-year post-vaccination timepoint, which suggests that the protective effect does not wane over time. The 100% efficacy against HPV 16/18-associated CIN2+ at year 11 was based on 34 CIN2+ events, all in the unvaccinated group, resulting in a lower CI bound of 89%, suggesting that the results are robust. Our findings show that the bivalent vaccine results in protection against CIN3, the immediate precursor of invasive cervical cancer. In our assessment of cumulative HPV vaccine efficacy, the two cases of CIN3 detected at years 1 and 4 in the HPV vaccine group might have originated from existing infections present before vaccination that were undetected during the vaccination phase. Even if the two cases were considered the result of true vaccine failures, the protection afforded by the vaccine has the potential to result in substantial cervical cancer reductions among HPV-vaccinated women.

Our findings showing the long-term protection offered by the bivalent HPV vaccine are supported by our previous reports of stable, high efficacy against HPV 16/18 prevalent

infection at year 11 and the high level of HPV 16 and HPV 18 antibodies persisting throughout the study.[20,21] Ongoing analyses will assess efficacy against CIN2+ irrespective of HPV type associated with the lesion. Our findings are consistent with one clinical trial of the bivalent vaccine done in China, in which significant protection against HPV 16/18-associated CIN2+ was reported for up to 6 years (90% efficacy).[9] Duration of protection of the bivalent vaccine was also assessed in a passive cancer registry-based follow-up study, which reported 66% protection against CIN3, 10 years after vaccination.[24] For the quadrivalent vaccine, reported vaccine effectiveness against HPV 16/18 CIN2+ has been shown to remain higher than 90% at 10 years post-vaccination.[25] Additionally, a meta-analysis of the population-level impact of HPV vaccination on CIN2+ occurrence showed a significant decrease in the prevalence of 51% in CIN2+ among screened girls aged 15–19 years and 31% in women aged 20–24 years, 5–9 years after vaccination.[26] Vaccine-induced antibodies are the known mediators of protection afforded by prophylactic HPV vaccines and nearly 100% of the women who received the vaccine and were assessed for antibody responses seroconverted and remained seropositive after 11 years, supporting the observation of robust and durable vaccine efficacy.[20,27]

Important strengths of our long-term follow-up study include the duration and high retention rates. Histological outcomes were determined by a panel of expert pathologists masked to treatment allocation, reducing misclassification and ensuring robust assessment of the primary endpoint by a panel of expert pathologists who blindly reviewed all slides. A substantial number of women developed CIN2+ during follow-up, increasing the precision of our efficacy estimates. The main limitation of our study was the replacement of the original control group (women offered HPV vaccination after completion of the year 4 visit), with a new unvaccinated group. As previously reported,[13] the new unvaccinated group was similar to the original control group in terms of risk of HPV acquisition, which is the precursor to cervical disease.[13] Moreover, our sensitivity analyses of the inclusive cohort, which provided a worst-case scenario, showed vaccine efficacy for the prevention of HPV 16/18-associated CIN2+ remained high at year 11.

Between years 7–11, no women in the HPV vaccine group developed CIN2+ despite continued disease detection in the unvaccinated group, which suggests that the vaccine offers prolonged protection against clinical disease.[28] It should be noted that these results apply to the bivalent HPV vaccine, which at the time of writing has had more limited distribution than the quadrivalent and nonavalent vaccines that are licensed.

Robust data showing that HPV vaccines provide durable protection against HPV 16/18 infections and associated precancerous lesions has continued to accumulate, supporting the notion that invasive cervical cancer is preventable.[29,30]

Contributors

CP, ARK, JNS, AH, RH, JS, and SHT formed the core analysis and writing team. CP, RH, ACR, and RO were responsible for the collection of field data. SW, JB, and WQ designed and oversaw the HPV genotyping assays. DG, TMD, and MHS provided the diagnosis of histological slides for outcome definition. All authors, including members of the Costa Rica HPV Vaccine Trial Group, qualified for authorship in adherence with the ICMJE guidelines and reviewed and commented upon a draft, gave final approval, and had final responsibility for the decision to submit for publication. All authors contributed towards study design, acquisition of data or statistical analyses, interpretation of data, and writing or finalising the manuscript.

Declaration of interests

TMD reports personal fees from BD, Roche, Antiva, and TheVax, outside the submitted work. DRL and JTS are named inventors on US Government-owned HPV vaccine patents that are licensed to GlaxoSmithKline and Merck and for which the National Cancer Institute receives licensing fees, and are both entitled to limited royalties as specified by federal law. All other authors declare no competing interests.

Data sharing

Participant data can be shared with outside collaborators for research to understand more about the performance of the HPV vaccine, immune response to the vaccine, and broader study factors associated with the natural history of HPV infection and risk factors for infection and disease. Outside collaborators can apply to access our protocols and data from the blinded phase of the Costa Rica Vaccine Trial (NCT00128661). Outside collaborators can apply for access to the data online. Data for the long term follow-up phase are not yet available. A trial summary, current publications, and contact information are available online.

For the **trial summary, current publications, and contact information for data access** see https://dceg.cancer.gov/research/who-we-study/cohorts/costa-rica-vaccine-trial

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