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Efficacy of the HPV-16/18 vaccine: Final according to protocol results from the blinded phase of the randomized Costa Rica HPV-16/18 vaccine trial

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ABSTRACT

Background: A community-based randomized trial was conducted in Costa Rica to evaluate the HPV-16/18 AS04-adjuvanted vaccine (NCT00128661). The primary objective was to evaluate efficacy of the vaccine to prevent cervical intraepithelial neoplasia 2 or more severe disease (CIN2+) associated with incident HPV-16/18 cervical infections. Secondary objectives were to evaluate efficacy against CIN2+ associated with incident cervical infection by any oncogenic HPVs and to evaluate duration of protection against incident cervical infection with HPV-16/18. Vaccine safety and immunogenicity over the 4-year follow-up were also evaluated.

Methods: We randomized (3727 HPV arm; 3739 control arm), vaccinated (HPV-16/18 or Hepatitis A) and followed (median 53.8 months) 7466 healthy women aged 18–25 years. 5312 women (2635 HPV arm; 2677 control arm) were included in the according to protocol analysis for efficacy. The full cohort was evaluated for safety. Immunogenicity was considered on a subset of 354 (HPV-16) and 379 (HPV-18) women. HPV type was assessed by PCR on cervical specimens. Immunogenicity was assessed using ELISA and inhibition enzyme immunoassays. Disease outcomes were histologically confirmed. Vaccine efficacy and 95% confidence intervals (95%CI) were computed.

Results: Vaccine efficacy was 89.8% (95% CI: 39.5–99.5; $N = 11$ events total) against HPV-16/18 associated CIN2+, 59.9% (95% CI: 20.7–80.8; $N = 39$ events total) against CIN2+ associated with non-HPV-16/18 oncogenic HPVs and 61.4% (95% CI: 29.5–79.8; $N = 51$ events total) against CIN2+ irrespective of HPV type. The vaccine had an acceptable safety profile and induced robust and long-lasting antibody responses.

Conclusions: Our findings confirm the high efficacy and immunogenicity of the HPV-16/18 vaccine against incident HPV infections and cervical disease associated with HPV-16/18 and other oncogenic HPV types. These results will serve as a benchmark to which we can compare future findings from the ongoing extended follow-up of participants in the Costa Rica trial.

Trial registration: : Registered with clinicaltrials.gov: NCT00128661.

Abbreviations: ATP, according to protocol; AE, adverse event; CI, confidence interval; CIN2+, cervical intraepithelial neoplasia 2 or more severe disease; CVT, Costa Rica HPV-16/18 vaccine trial; GMT, geometric mean titer; HPV, human papillomavirus; DEIA, SPF_{10} HPV DNA enzyme immunoassay; EIA, inhibition enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; NCI, National Cancer Institute; SAE, serious adverse event; VE, vaccine efficacy; VLP, virus-like particle.

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1. Introduction

Three programs launched in the 2000s evaluated prophylactic virus-like particle (VLP) human papillomavirus (HPV) vaccines [1–3]. Two of these programs were led by manufacturing companies, Merck Pharmaceuticals and GlaxoSmithKline Biologicals, who licensed the HPV-VLP technology and developed vaccines to prevent cervical cancers caused by HPV-16 and HPV-18, two HPV types that account for up to 70% of cervical cancers worldwide. Results from trials by these companies demonstrated that the vaccines have an acceptable safety profile and are highly effective for the prevention of HPV infections and lesions associated with vaccine types, and in some instances to additional, related types. As a result, both vaccines are licensed for use in adolescents and young adults in many countries [4–10].

The third program initiated pre-licensure was a community-based trial in Costa Rica (NCT00128661) sponsored by the US National Cancer Institute (NCI) that utilized the HPV-16/18 AS04-adjuvanted vaccine (*Cervarix*®, hereafter referred to as the HPV-16/18 vaccine) provided to NCI by GlaxoSmithKline Biologicals [11]. This trial was designed to evaluate the efficacy, safety, impact and immune mechanisms associated with HPV vaccination, and to extend natural history studies to vaccinated groups. To date, results from the Costa Rica HPV-16/18 vaccine trial (CVT) have shown that (1) the vaccine is highly effective at preventing new persistent infections with HPV-16/18 [12], (2) the vaccine confers partial protection against HPV types phylogenetically related to HPV-16/18 [13], (3) the vaccine does not help treat infections [14], (4) fewer than 3 doses of the vaccine appear to protect as well as the full 3-dose series for at least 4 years against persistent HPV-16/18 infections [15], (5) there are indications that the vaccine protects against HPV infection at the anus and oral cavity [16,17], (6) the vaccine does not impact overall rates of pregnancies/pregnancy outcomes [18], (7) the impact of vaccination declines with increasing age at vaccination [12], (8) the initial impact of young adult vaccination on colposcopy referral/treatment rates in well-screened populations are modest [19], (9) within the same age group, levels of antibodies achieved long-term following two doses (0 and 6 months) of the vaccine are high and only slightly lower than those observed after three doses and antibodies achieved long-term following one dose of the vaccine are lower than those observed with 3 doses but stable [20], (10) vaccination induces cross-neutralizing potential in sera of vaccinees [21], and that (11) modest antibody levels generated by natural HPV infection provide partial protection against re-infection [22].

We now extend those findings by presenting results from the blinded analysis conducted at the end of the first four years of follow-up. These results focus on the according to protocol (ATP) efficacy findings submitted to the FDA under BB-IND #7920; separate submissions focus on findings from intent-to-treat and naïve analyses from our trial [12,23].

2. Materials and methods

2.1. Design, subjects, procedures, and testing

This analysis presents a double-blind randomized controlled trial of an HPV-16/18 vaccine among healthy women 18–25 years old. The study was approved by the Institutional Review Boards in Costa Rica and the US. Detailed methods have been published [11]. In brief, potential participants from a census were invited between June 2004 and December 2005. Eligible women who agreed to participate ($N = 7466$; estimated to provide >80% power to observe expected differences between arms) were randomized with equal chance to the HPV-16/18 (HPV arm) or Hepatitis A vaccine (control arm), offered in three doses over approximately six months. Blinding to arm assignment was maintained throughout the 48-month follow-up and until the analytic datafile was frozen.

At enrollment, a pelvic exam was performed on sexually experienced women. Exfoliated cells were collected for cytology, HPV DNA, and other tests. At the 6-month visit, women were asked to provide a self-collected cervical specimen for HPV testing. Blood was collected from participants. Each participant was scheduled for annual follow-up examinations (median follow-up time = 53.8 months; inter-quartile range: 50.5–57.0), at which time a pelvic examination was performed on sexually active women, and exfoliated cells and blood were collected. On a pre-defined subset, an additional visit approximately one month following the last vaccine dose was performed where blood was collected for immunological assessment.

Cytology was classified using the Bethesda system. Women with low-grade squamous intraepithelial lesions (LSIL) or HPV positive atypical squamous cells of undetermined significance (ASC-US) were followed semi-annually. The colposcopy referral algorithm used in our trial parallels that used for the PATRICIA trial [6]. Specifically, a repeat LSIL/HPV positive ASC-US, an ASC-US-rule out high-grade SIL (ASC-H), high-grade squamous intraepithelial lesions or more severe disease (HSIL+), or glandular abnormalities prompted colposcopy and treatment as needed [11].

HPV testing using the Hybrid Capture 2 test was performed on enrollment specimens plus specimens from women with an ASC-US cytology during follow-up for clinical management [11]. Broad spectrum PCR-based HPV DNA testing was performed on specimens based on amplification and broad spectrum probe hybridization using the SPF_{10} HPV DNA enzyme immunoassay system followed by typing using the $LiPA_{25}$ version 1 line detection system and HPV-16 and -18 type specific testing [11]. Enzyme-linked immunosorbent assay (ELISA) was used to detect and quantify IgG antibodies against HPV-16 and -18 in the subset of women selected for the extra visit one month after the last vaccination dose [4,24]. Immunogenicity was also assessed by a V5/J4 monoclonal antibody inhibition enzyme immunoassay (EIA), which in contrast to the ELISA detects specific neutralizing epitopes [24,25].

2.2. Objectives

The primary objective was to evaluate efficacy of the vaccine to prevent cervical intraepithelial neoplasia 2 or more severe disease (CIN2+) associated with incident (post dose 3) HPV-16/18 cervical infections. Secondary objectives were to evaluate efficacy to prevent CIN2+ associated with incident cervical infection by any oncogenic HPV type and to evaluate the duration of protection conferred by the vaccine against incident cervical infection with HPV-16/18. Vaccine safety and immunogenicity over the 4-year follow-up were also evaluated.

2.3. Analytical cohorts

The cohort for efficacy analyses included subjects who received three doses within protocol-defined windows, whose timing between doses was respected (21–90 days between doses 1 and 2; 90–210 days between doses 2 and 3), who were HPV DNA negative at Months 0 and 6 for the HPV type considered in the analysis, who did not have a biopsy or treatment (loop electrosurgical excisional procedure) during the vaccination phase, for whom there was no investigational new drug safety report during the vaccination period, and who otherwise complied with the protocol during the vaccination period (Fig. 1). The cohort for safety was defined as subjects who received at least one dose of vaccine and therefore represents the intention to treat cohort ($N = 7466$). The cohort for

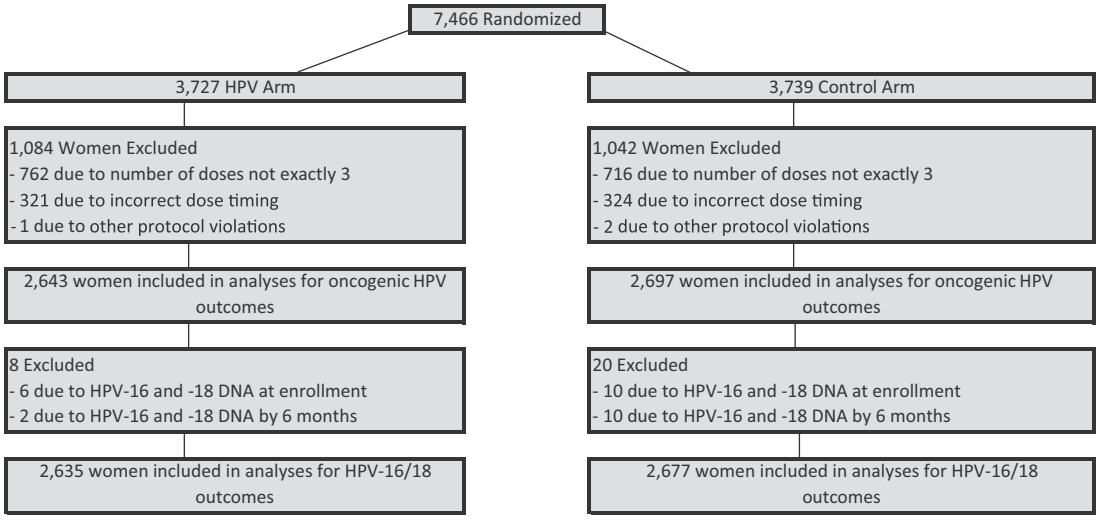


Fig. 1. Consort diagram – analytic cohort for efficacy analyses.

immunogenicity was defined as subjects included in the immunogenicity subcohort who met the criteria defined for the efficacy cohort above and whose timing between the third vaccine dose and the extra visit was 30–60 days ($N = 354$ women for HPV-16 analysis; $N = 379$ for HPV-18 analysis).

2.4. Outcomes

The primary outcome for efficacy was defined as histopathologically confirmed CIN2+ associated with HPV-16/18 cervical infection detected by PCR in the cervical cytology specimen that led to colposcopy referral. Final histological diagnosis was defined based on blinded review by a Costa Rican and a US pathologist, with blinded review by a third pathologist in instances where the first two reviewers disagreed [11]. In secondary efficacy analyses, we evaluated histopathologically confirmed CIN2+ associated with non-HPV-16/18 and any oncogenic HPV cervical infections (HPV types 16,18,31,33,35,39,45,51,52,56,58,59,68/73) detected by PCR in the cervical cytology specimen that led to colposcopy referral, and time to incident infection with HPV-16/18 cervical infections. In exploratory efficacy analyses, an alternative (referred to hereafter as "exploratory" to distinguish it from the a priori definition described above) definition of HPV type attribution to CIN2+ lesions was used that considered evidence of HPV persistence preceding referral to colposcopy when attributing HPV types to lesions in instances when >1 HPV type was present in the cervical cytology specimen that led to colposcopy referral. We also evaluated histopathologically confirmed CIN2+, irrespective of HPV type, in an analysis that considered outcomes that occurred in the absence of HPV during the vaccination period.

For safety analyses, solicited local and general adverse events (AEs) within 60 min after vaccination (all subjects) or from day 3–6 post-vaccination (10% random subset) were evaluated. Unsolicited AEs, serious adverse events (SAEs), and pregnancies/pregnancy outcomes were documented throughout the 4-year study period. Impact of vaccination on pregnancies/pregnancy losses was reported on separately [18] and is not considered here because limited new blinded information on pregnancies around vaccination was accrued after the initial report.

For immunogenicity analyses, we evaluated presence and level of HPV-16 and HPV-18 antibodies by ELISA and by HPV-16 V5 and HPV-18 J4 monoclonal antibody inhibition EIA measured during the vaccination period, at one month after the last vaccination, and at annual visits thereafter in the subjects enrolled into the immunogenicity cohort.

2.5. Statistical analysis

Vaccine efficacy (VE), defined as the percentage reduction in an endpoint due to the vaccine, was estimated as the complement of the ratio of the attack rates (risk ratio) in the HPV and control arms. The attack rate was calculated as the percentage of women who experienced the endpoint. The complement of the 95% confidence interval (95% CI) for the risk ratio was used to calculate the CI for the VE estimates. The difference between the attack rates in the two arms was used to assess rate reductions. The CI for the difference was calculated using the conditional exact test. Separate analyses were conducted for HPV-16/18, all oncogenic HPV types combined, all oncogenic HPV types combined excluding HPV-16/18, individual HPV types, and irrespective of HPV type.

The proportion of subjects with at least one SAE classified by International Classification of Diseases Version 10 during the study is presented by study group. Similar information is presented for grade 3 (severe) SAEs and for SAEs classified by the local investigator as possibly related to vaccination. We report separately the proportion of subjects with at least one reported autoimmune AE, neurological AE or death.

Seropositivity rates and Geometric Mean Titers (GMTs) with 95% CIs were calculated. When calculating GMTs, antibody titers below the assay cut-off were given a value of half the cut-off.

3. Results

Participants in the HPV and control arms of the trial and included in the ATP cohort for efficacy were comparable with respect to age, clinic, sexual behavior and HPV-16/18 serology and DNA results at entry (Supplemental Table 1).

Supplementary Table 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.06.038.

Number of CIN2+ events, rates and efficacy are presented in Table 1. Efficacy against incident HPV-16/18 associated CIN2+ was 89.8% (95% CI = 39.5–99.5; rate reduction = 3.4/1000 women) using our a priori algorithm for HPV type attribution and 88.7% (95% CI = 31.3–99.5; rate reduction = 3.0/1000 women) using the alternative (exploratory) definition that considers viral persistence when

Table 1
Vaccine efficacy against CIN2+ outcomes – ATP cohort for efficacy – Costa Rica HPV-16/18 vaccine trial (CVT).[a]

HPV type	Arm	Women in ATP cohort, *N*	Women with CIN2+ events, *n*	Rate (per 1000 women)	Rate Reduction (95% CI[a])	Efficacy, % (95% CI[a])
HPV-16/18	HPV	2635	1	0.4		
	Control	2677	10	3.7	3.4 (1.0, 4.1)	89.8 (39.5, 99.5)
HPV-16/18 (exploratory)	HPV	2635	1	0.4		
	Control	2677	9	3.4	3.0 (0.7, 3.7)	88.7 (31.3, 99.5)
Non-HPV-16/18 oncogenic	HPV	2643	11	4.2		
	Control	2697	28	10.4	6.2 (1.7, 9.8)	59.9 (20.7, 80.8)
Non-HPV-16/18 oncogenic (exploratory)	HPV	2643	5	1.9		
	Control	2697	24	8.9	7.0 (3.3, 9.3)	78.7 (47.1, 92.8)
Oncogenic HPV	HPV	2643	11	4.2		
	Control	2697	33	12.2	8.1 (3.4, 11.7)	66.0 (34.0, 83.5)
Oncogenic HPV (exploratory)	HPV	2643	5	1.9		
	Control	2697	29	10.8	8.9 (5.1, 11.2)	82.4 (57.0, 94.0)

[a] CIN2+, Cervical intraepithelial neoplasia 2 or more severe disease; ATP, according to protocol; 95% CI, 95% confidence interval.

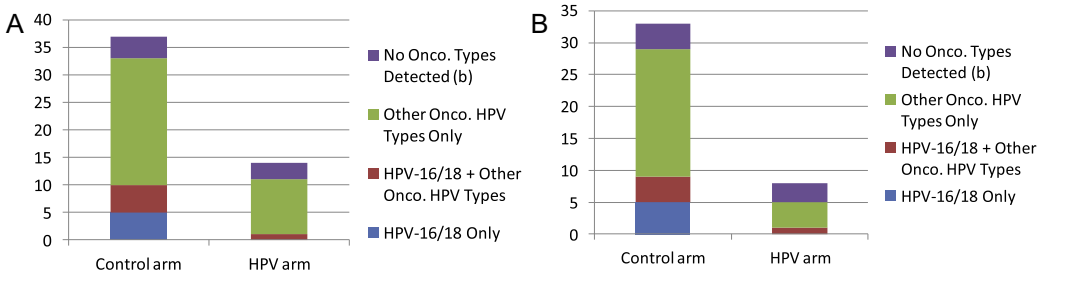
making HPV type attribution. A total of 11 HPV-16/18 associated CIN2+ events were observed using our a priori definition; 10 were CIN2 and one was a CIN3. The single HPV-16/18 CIN2+ event in the HPV arm occurred in a participant who at entry had antibodies against both HPV-16 and HPV-18, and evidence (by DNA test) of infection with a non-oncogenic HPV type (HPV-66), and who was positive (by DNA test) for HPV-16 and -45 11 months after enrollment and diagnosed with CIN3 15 months after enrollment. Efficacy estimates against CIN2+ associated with non-HPV-16/18 oncogenic HPV types were 59.9% (a priori definition) and 78.7% (exploratory definition). The breakdown of HPV types detected by arm is summarized in Fig. 2a (a priori definition) and b (exploratory definition). Efficacy estimates irrespective of HPV type were 61.4% (95% CI = 29.5–79.8; rate reduction = 8.4/1000 women; *N* = 37 in control arm and 14 in HPV arm) by our a priori and 75.3% (95% CI = 48.1–89.3; rate reduction = 9.2/1000 women; *N* = 33 in control arm and 8 in HPV arm) by our exploratory definition of incident outcomes. Results for individual oncogenic HPV types are summarized in Supplemental Tables 2a and 2b.

Supplementary Tables 2a and 2b related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.06.038.

Efficacy against incident HPV-16/18 infections during the study was 79.5% (95% CI = 74.0–84.0; rate reduction = 115/1000 women) (Table 2). Efficacy in this group of young adults was lowest in the first year of follow-up (57.1%; 95% CI = 33.2–73.0) and higher in subsequent years (82.6% in year 4+; 95% CI = 73.0–89.2).

Safety findings are summarized in Table 3. Rates of solicited local and general AEs were comparable in the two arms in the hour following vaccination. The rate of local solicited AEs within 3–6 days following any vaccination was higher among those in the HPV arm (53.7% for all; 1.8% for grade 3 AEs) compared to the control arm (19.9% for all; 0.0% for grade 3 AEs). Unsolicited AEs reported in the month following any vaccination were comparable between arms. The proportion of participants with SAEs, SAEs possibly related to vaccination, medically significant conditions, new-onset chronic diseases, autoimmune AEs, neurological AEs, and deaths were comparable between arms. All but 12 SAEs possibly related to vaccination were pregnancy related [18]. For the 12 remaining SAEs possibly related to vaccination, 7 occurred in the HPV arm (1 Crohn's disease, 1 ulcerative colitis, 1 rheumatoid arthritis, 1 haematuria, 1 thyrotoxicosis, 1 excessive and frequent menstruation with irregular cycle, and 1 somatoform autonomic dysfunction) and 5 in the control arm (2 anaphylactic shock events, 1 generalized skin eruption, 1 acute appendicitis, and 1 unspecified abnormality of gait/mobility). The 43 autoimmune events were equally distributed across arms (22 in HPV arm; 21 in control arm) and were due to goiter (8 in HPV arm; 9 in control arm), rheumatoid arthritis (4 in HPV arm; 6 in control arm), inflammatory bowel disease (3 in HPV arm including 1 Crohn's disease; 2 in control arm), systemic lupus erythematosus (2 in HPV arm; 1 in control arm), insulin-dependent diabetes mellitus (1 in HPV arm; 1 in control arm) and other conditions (4 in HPV arm; 2 in control arm). The 15 deaths observed were equally distributed across arms (8 in HPV arm; 7 in control arm) and were due to suicides (4 in control arm), automobile accidents (1 in HPV arm; 2 in control arm), physical assault (2 in HPV arm), cancer (1 in HPV arm; 1 in control arm), Crohn's disease (1 in HPV arm), systemic lupus erythematosus (1 in HPV arm), HIV-associated conditions (1 in HPV arm), and acute myocardial infarction (1 in HPV arm).

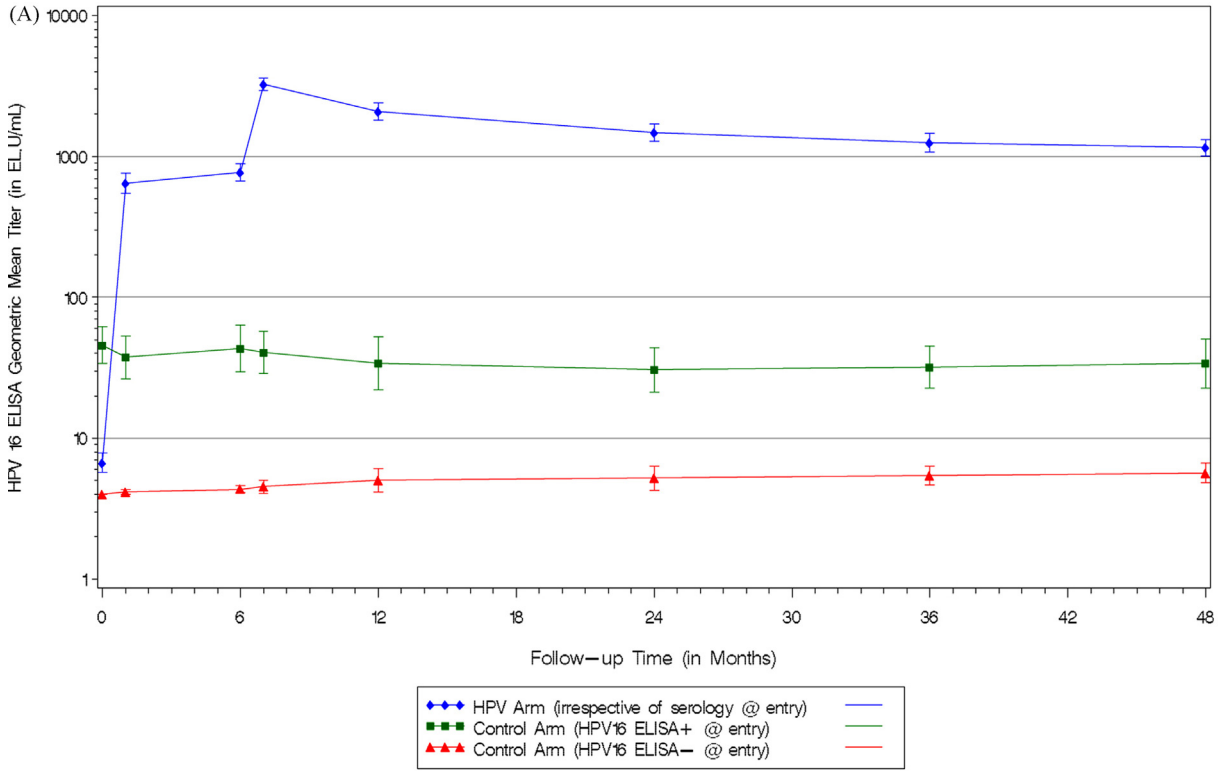
Immunogenicity results are summarized in Fig. 3a–d. GMTs peaked at one month following last dose, declined thereafter and



[a] CIN2+ = Cervical intraepithelial lesion or more severe disease; HPV type attribution for a-priori outcome defined based on HPV type(s) detected in the cytology specimen that led to colposcopy referral.
[b] Only non-oncogenic incident HPV types were detected for three women, positivity for uncharacterized HPV types was observed for two women, and no HPV was detectable for two women.

[a] CIN2+ = Cervical intraepithelial lesion or more severe disease; HPV type attribution for exploratory outcome defined considering evidence of HPV persistence preceding colposcopy when multiple HPV types were present in the cytology specimen that led to colposcopy referral.
[b] Only non-oncogenic incident HPV types were detected for three women, positivity for uncharacterized HPV types was observed for two women, and no HPV was detectable for two women.

Fig. 2. (a) Distribution of CIN2+ outcomes by HPV type (a priori) and vaccination arm – according to protocol cohort for efficacy – Costa Rica HPV-16/18 vaccine trial.[a] (b) Distribution of CIN2+ outcomes by HPV Type (strict) and vaccination arm – according to protocol cohort for efficacy – Costa Rica HPV-16/18 vaccine trial.[a]



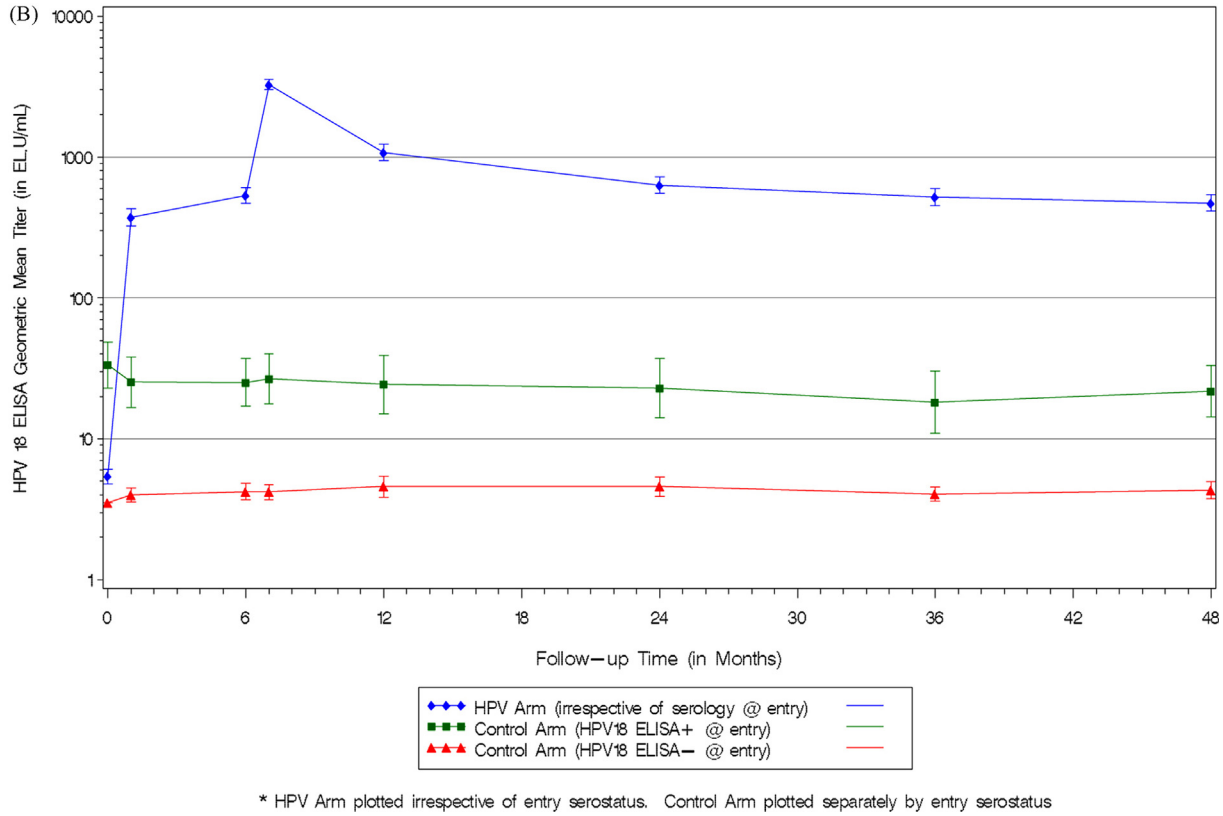
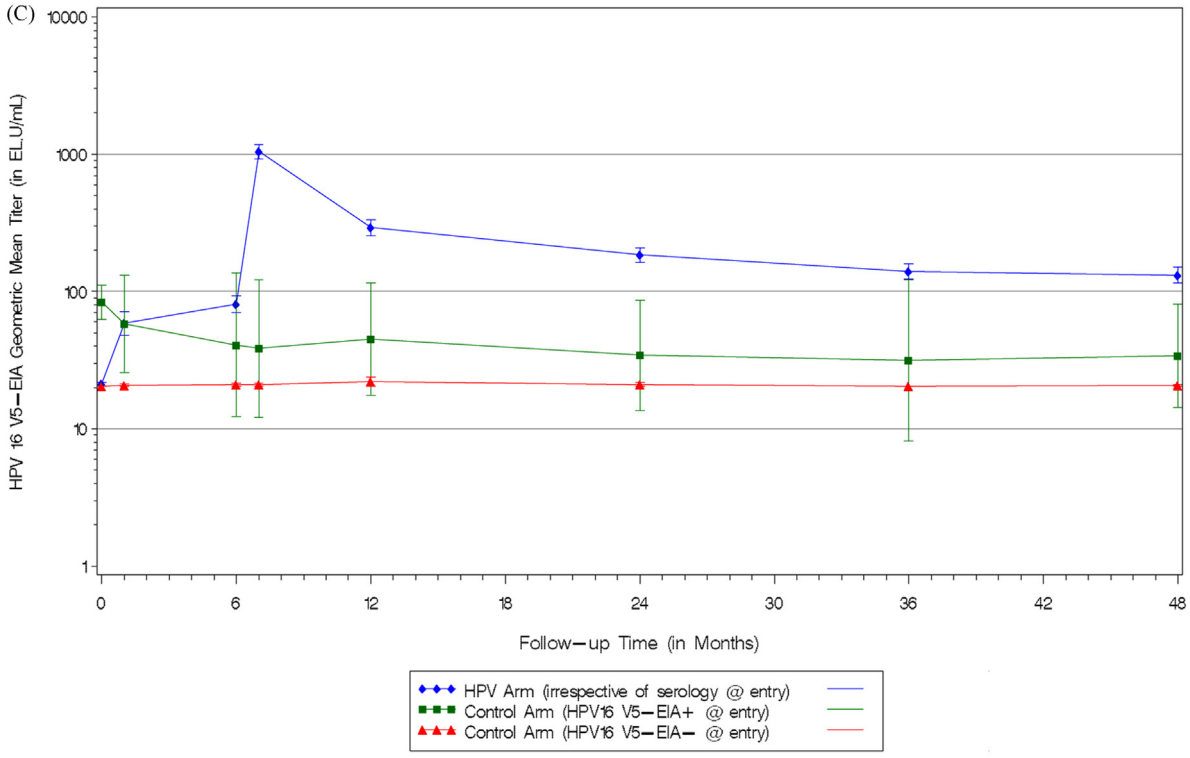
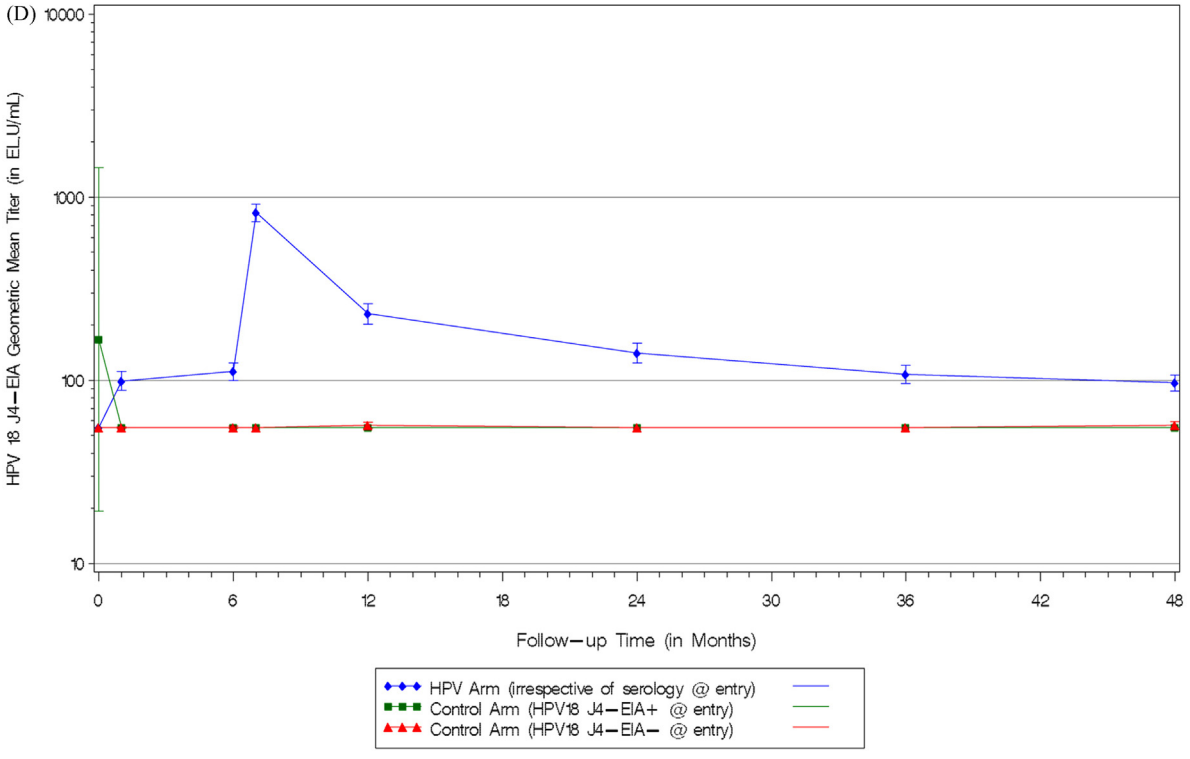


Fig. 3. (a) Kinetics of HPV-16 antibody response by vaccination arm.* (ELISA assay) – immunogenicity subcohort – Costa Rica HPV-16/18 vaccine trial (CVT). (b) Kinetics of HPV-18 antibody response by vaccination arm.* (ELISA assay) – immunogenicity subcohort – Costa Rica HPV-16/18 vaccine trial (CVT). (c) Kinetics of HPV-16 antibody response by vaccination arm.* (V5–EIA assay) – immunogenicity subcohort – Costa Rica HPV-16/18 vaccine trial (CVT). (d) Kinetics of HPV-18 antibody response by vaccination arm.* (J4 – EIA assay) – immunogenicity subcohort – Costa Rica HPV-16/18 vaccine trial (CVT).



* HPV Arm plotted irrespective of entry serostatus. Control Arm plotted separately by entry serostatus



* HPV Arm plotted irrespective of entry serostatus. Control Arm plotted separately by entry serostatus

Fig. 3. (*Continued*).

Table 2
Vaccine efficacy against incident HPV-16/18 detection by time since first vaccination – ATP cohort for efficacy – Costa Rica HPV-16/18 vaccine trial (CVT).[a]

Time	Arm	Women in ATP cohort, *N*	Women with HPV-16/18 events, *n*	Rate (per 1000 women)	Rate reduction (95% CI[a])	Efficacy, % (95% CI[a])
Overall	HPV	2635	78	29.6		
	Control	2677	387	144.6	115.0 (102.3, 126.1)	79.5 (74.0, 84.0)
Year 1	HPV	2380	27	11.3		
	Control	2420	64	26.4	15.1 (7.5, 21.7)	57.1 (33.2, 73.0)
Year 2	HPV	2313	18	7.8		
	Control	2349	117	49.8	42.0 (34.5, 47.8)	84.4 (74.8, 90.7)
Year 3	HPV	2232	11	4.9		
	Control	2166	88	40.6	35.7 (29.1, 40.3)	87.9 (78.0, 93.8)
Year 4+	HPV	2421	22	9.1		
	Control	2261	118	52.2	43.1 (35.0, 49.5)	82.6 (73.0, 89.2)

[a] ATP, according to protocol; 95% CI, 95% confidence interval.

remained relatively stable beyond 12–24 months post-vaccination. By ELISA, we observed that 100% of vaccinated participants were seropositive against HPV-16 and HPV-18 after three doses and remained seropositive at the end of the 4-year follow-up period. By EIA, we observed that 100% and 99.5% of vaccinated participants were seropositive against HPV-16(V5) and HPV-18(J4), respectively, after three doses. At the end of the 4-year follow-up period, 92.3% and 45.8% of vaccinated participants remained seropositive against HPV-16(V5) and HPV-18(J4), respectively.

4. Discussion

This report summarizes results from the final ATP analysis of the NCI-sponsored CVT under GlaxoSmithKline Biologicals'

Table 3
Summary of safety outcomes – total vaccinated cohort[a] – Costa Rica HPV-16/18 vaccine trial (CVT).

	HPV arm		Control arm	
	n	%	*n*	%
Within 60 min following any vaccination (N = 3727 HPV & 3739 control arms)				
Any solicited adverse event				
All	2534	68.0	2519	67.4
Grade 3	32	0.9	27	0.7
Solicited local adverse events[b]				
All	1918	51.5	1902	50.9
Grade 3	25	0.7	22	0.6
Solicited general adverse events[c]				
All	1580	42.4	1543	41.3
Grade 3	8	0.2	6	0.2
Within day 3–6 following any vaccination (N = 380 HPV & 376 control arms; 10% sample)				
Any solicited adverse event				
All	358	94.2	339	90.2
Grade 3	11	2.9	2	0.5
Solicited local adverse events[b]				
All	204	53.7	75	19.9
Grade 3	7	1.8	0	0.0
Solicited general adverse events[c]				
All	344	90.5	335	89.1
Grade 3	4	1.1	2	0.5
Within 30 days following any vaccination (N = 3727 HPV & 3739 control arms)				
Unsolicited adverse events				
All	1638	43.9	1536	41.1
Grade 3	34	0.9	30	0.8
Serious adverse events during entire study period (N = 3727 HPV & 3739 control arms)				
Any serious adverse events	912	24.5	891	23.8
Serious adverse events possibly related to vaccination	53	1.4	39	1.0
Medically significant conditions[d]	744	20.0	739	19.8
New-onset chronic disease[d]	383	10.3	417	11.2
Autoimmune adverse events[d]	22	0.6	21	0.6
Neurological adverse events[d]	626	16.8	591	15.8
Deaths	8	0.2	7	0.2

[a] Four participants who erroneously received both vaccine types were assigned to their original randomization arm (1 HPV arm; 3 control arm). These individuals reported no serious adverse events.
[b] Solicited local adverse events included pain, redness and swelling.
[c] Solicited general adverse events included fatigue, myalgia, arthralgia, gastro-intestinal symptoms, headache, rash, urticaria, and fever.
[d] Medically significant conditions were defined as grade 3 (severe) serious adverse events. As described previously [36], all adverse events reported during the trial were compared with a pre-defined list of potential chronic diseases derived from the medical dictionary for regulatory activities. Determination of whether a chronic disease was of new onset was based on blinded review of the reported symptoms and the subject's pre-vaccination medical history. A separate list, restricted to potential autoimmune events (e.g. systemic lupus erythematosus, thyroiditis), which excluded allergy-related events or isolated signs and symptoms and events not considered to be autoimmune in origin, was used to identify new onset autoimmune diseases among events identified as new onset chronic diseases. Neurologic adverse events are all preferred terms belonging to the system organ class 'nervous system disorders'.

Table 4
Summary of findings from randomized clinical trials of prophylactic virus-like particle human papillomavirus vaccines.

	Bivalent vaccine trials (HPV-16/18 AS04-adjuvanted vaccine)			Quadrivalent vaccine trials (HPV-6/11/16/18 vaccine)
	Costa Rica vaccine trial (CVT) Phase III; NCT00128661	Japan efficacy trial [26] Phase II; NCT00316693/NCT00929526	PApilloma TRIal against cancer in young adults (PATRICIA) [6,28] Phase III; NCT00122681	Pooled analysis from 2 to 3 studies [9,37–39]: -007: phase II; NCT n/a -013 (FUTURE I): phase III; NCT00092521 -015 (FUTURE II): phase III; NCT00092534
Countries	Community-based trial (Guanacaste; Costa Rica)	Japan	Multi-country trial (Australia, Belgium, Brazil, Canada, Finland, Germany, Italy, Mexico, Philippines, Spain, Taiwan, Thailand, UK, and USA).	Multi-country trials (Australia, Austria, Brazil, Canada, Colombia, Czech Republic, Denmark, Finland, Germany, Hong Kong, Iceland, Italy, Mexico, New Zealand, Norway, Peru, Poland, Puerto Rico, Russia, Singapore, Sweden, Thailand, UK, USA)
Age	18–25 years	20–25 years	15–25 years	16–26 years
Total enrolled subjects	7466	1046	18,729	18,174 (protocols 007, 013 and 015)
Analytical cohort	ATP cohort for efficacy: received 3 doses within protocol defined windows, HPV DNA negative for the HPV type under consideration at months 0 and 6, no biopsy or treatment (loop electrosurgical excisional procedure) during the vaccination phase, no protocol violation	ATP cohort for efficacy: received 3 doses within protocol defined windows, HPV DNA negative for the HPV type under consideration at months 0 and 6, seronegative for the HPV type under consideration at month 0, normal or low-grade cytology at month 0, no protocol violation		Per-protocol: received 3 doses within 1 year, PCR negative and seronegative to HPV-6, HPV-11, HPV-16, or HPV-18 at enrolment, remained PCR negative to the same vaccine HPV type (s), to which they were naive at enrolment, through 1 month post-dose 3, no protocol violation
Endpoints	CIN2+: CIN2, CIN3, adenocarcinoma in situ or invasive carcinoma	CIN2+: CIN2, CIN3, adenocarcinoma in situ or invasive carcinoma	CIN2+: CIN2CIN3, adenocarcinoma in situ or invasive carcinoma	CIN2 or worse
Follow-up	48 months post-dose 1	48 months post-dose 1	48 months post-dose 1	42 months post-dose 1

Efficacy estimates

HPV16/18 types	Current manuscript		Konno et al., 2014 [26]		Lehtinen et al., 2012 [6]		Kjaer et al., 2009 [37]	
	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control
N	2635	2677	406	404	7338	7305	7864	7865
Cases	1	10	0	5	5	97	2	110
Efficacy (95% CI)	88.7% (31.3, 99.5)[a]		100% (−8.0, 100)		94.9% (87.7, 98.4)		98.2% (93.3, 99.8)[b]	

Non-vaccine oncogenic types	HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68/73 [Current manuscript]		HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68/73 [26]		HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68/73 [28]		HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, -59 [38,39]	
	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control
N	2643	2697	444	435	8067	8047	4616	4680
Cases	5	24	4	12	88	165	62	93
Efficacy (95% CI)	78.7% (47.1, 92.8)[a]		67.7% (−6.6, 92.4)[c]		46.8% (30.7, 59.4)		32.5% (6.0, 51.9)[d]	

Irrespective of HPV	Current manuscript		Konno et al., 2014 [26]		Lehtinen et al., 2012 [6]		Munoz et al., 2010 [9]	
	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control
N	2643	2697	254	251	5466	5642	4616	4680
Cases	8	33	3	11	61	172	77	136
Efficacy (95% CI)	75.3% (48.1, 89.3)[a]		73.9% (1.1, 95.3)[e]		64.9% (52.7, 74.2)[e]		42.7% (23.7, 57.3)[d]	

ATP, according to protocol; CI, confidence interval; CIN2+, cervical intraepithelial neoplasia 2 or more severe disease; n/a, not available; *N*, number of women in each arm considered in the analysis; TVC, total vaccinated cohort.

[a] Vaccine efficacy using exploratory definition described in Section 2.

[b] Vaccine efficacy against HPV-6/11/16/18–related types for the quadrivalent vaccine.

[c] Vaccine efficacy in the ATP cohort for efficacy, regardless of baseline serostatus.

[d] Vaccine efficacy restricted to subjects who received ≥1 vaccine dose and, at enrolment, were HPV DNA negative for vaccine and nonvaccine types (31, 33, 35, 39, 45, 51, 52, 56, 58 and 59), seronegative for HPV-6, -11, -16 and -18, and had normal cytology.

[e] Vaccine efficacy in the TVC-naive cohort, i.e. women who received ≥1 vaccine dose and, at baseline, were HPV DNA negative for vaccine and nonvaccine types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68/73), seronegative for HPV-16 and -18, and had normal cytology.

FDA-BB-IND-7920. Our results confirm the high efficacy of VLP-based vaccines against incident CIN2+ associated with HPV-16/18 [4–10]. It is reassuring that high efficacy against infections and lesions associated with the HPV types in the vaccine formulation has now been reported for VLP-based vaccines from multiple trials conducted in different populations, despite differences in study methodology [4–10,26,27] (Table 4). Furthermore, our report is consistent with previous results suggesting that vaccination with the HPV-16/18 vaccine might confer partial protection against some oncogenic HPV types not included in the vaccine formulation [28]. We observed 60% efficacy against CIN2+ associated with incident oncogenic HPV infections with types other than HPV-16/18, an effect that increased to near 80% when we considered evidence of HPV persistence preceding referral to colposcopy. Although limited by small numbers, our findings suggest some efficacy (point estimates ranging from 42% to 100%) for all HPV types phylogenetically related to HPV-16 (A9 species – including HPV types 31,33,35,52,58). For HPV types phylogenetically related to HPV-18 (A7 species – including HPV types 39,45,59,68), evidence was mixed, with suggestion for efficacy against HPV-68 (which in our testing system was indistinguishable from non-oncogenic HPV-73) but not for other types related to HPV-18. Finally, when CIN2+ cases were examined irrespective of HPV type, we observed over 60% efficacy, an effect that increased to >75% when our exploratory criteria were used to define incident outcomes. It is important to note that such estimates of overall efficacy are likely to be population specific and to vary depending on the proportion of infections in the population attributable to vaccine types, non-vaccine HPV types for which there is cross-protection, and non-vaccine HPV types for which there is no cross-protection. In fact, vaccine efficacy against non-vaccine types or irrespective of HPV type reported from phase III randomized clinical trials to date have varied considerably as summarized in Table 4. It is not fully understood to what extent these observed differences are due to differences in study design and analysis (e.g. differences in colposcopy algorithm, sensitivity/specificity of HPV assays, and analytical cohorts evaluated), chance (95% confidence intervals tend to overlap), population differences (e.g. differences in relative distribution of non-vaccine HPV types in different study populations), or vaccine differences (i.e. real differences in cross protection between the bivalent and quadrivalent vaccines). In a recent evaluation of this issue, we have noted that differences observed in efficacy estimates between FUTURE I/II and PATRICIA are likely explained by a combination of these various factors [23].

We saw no evidence of waning efficacy during the study period. When we evaluated efficacy against HPV-16/18 infection over time, high efficacy (>80%) was observed in years 2–4+ and the lowest efficacy estimate was observed in the first year of follow-up (57%). The high efficacy observed in the out years is consistent with evidence of long-term protection up to 8.4 years (HPV-16/18 vaccine) and 5 years (HPV-6/11/16/18 vaccine) in the pharmaceutical trials [29,30]. We interpret the somewhat reduced efficacy in year 1 as suggestive that some outcomes might have resulted from undetected infections present before vaccination in our group of largely sexually experienced women [12].

The safety and immunogenicity profile of VLP-based vaccine have been evaluated in large-scale trials and results suggest that that vaccine has an acceptable safety profile, is generally well tolerated, and induces a robust and sustained immune responses [7,30–35]. Safety results from our trial are consistent with these previous reports. Similarly, consistent with previous reports, we noted robust antibody levels (measured by ELISA) following vaccination that persisted throughout the four years of follow-up – 100% of participants evaluated were seropositive against HPV-16 and HPV-18 at the end of follow-up. This is consistent with the high clinical efficacy observed. By the EIA inhibition assay that targets neutralizing epitopes for HPV-16 and HPV-18, we also observed robust responses following vaccination. These responses were measurable after four years for nearly all participants evaluated for HPV-16 (92.3%) and for roughly half of participants evaluated for HPV-18 (45.8%). Since efficacy remained high throughout the four years of follow-up for both HPV-16/18, the fact that about half of the vaccinees sero-reverted to HPV-18 by the EIA assay suggests that protective levels are lower than the minimum detectable level by the assay or that antibodies against additional epitopes can also be protective.

Limitations of our trial include the modest number of CIN2+ events among women naïve to specific HPV types during the vaccination period, which limited our ability to evaluate efficacy against individual HPV types other than HPV-16/18 and against CIN3+. Our study size also limited the ability to evaluate efficacy against lesions by time.

A distinguishing characteristic of our trial is its community-based design; we enrolled women from a well-defined area based on a census [11]. As a result, our trial represents a unique large-scale community-level trial conducted pre-licensure and affords an opportunity for follow-up studies to address many questions of interest. These include questions regarding long-term safety, immunogenicity and efficacy; natural history of infections in vaccinated women and the impact of vaccination on cervical disease associated with non-vaccine HPV types; the impact of vaccination on screening; and the utility of novel screening tools in vaccinated populations. The results presented herein serve as a benchmark to help interpret results from some of these planned efforts.

Our findings provide additional independent evidence of the efficacy, immunogenicity and safety of the HPV-16/18 vaccine for prevention of HPV infections and cervical cancer precursor lesions in previously unexposed women and further support the establishment of vaccination programs that target individuals prior to exposure.

Note: *Cervarix* is a registered trademark of the GlaxoSmithKline group of companies.

5. Investigators in the Costa Rica vaccine trial (CVT) group

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Conflicts of interest

F.S., G.C. and G.D. are employees of the GlaxoSmithKline group of companies. G.D. and F.S. receive stock options/restricted shares from the GlaxoSmithKline group of companies, and G.D. has previously received patent royalties from Wyeth Vaccines. The other authors declare that they have no conflicts of interest. The NCI receives licensing fees for HPV vaccines.

Author contribution

A.H. (NCI principal investigator), S.W. (NCI statistician) and R.H. (Costa Rica principal investigator) were responsible for the design and conduct of the study. From GlaxoSmithKline Vaccines, G.D. contributed to discussions regarding trial design and conduct. G.C. contributed toward data analyses and interpretation, and prepared the statistical analysis report submitted to the FDA. F.S. and G.D. critically reviewed the study report in close collaboration with NCI and Costa Rica co-principal investigators. A.H. wrote the manuscript, and all other authors reviewed and commented on the initial and subsequent drafts. All authors had full access to the data and gave final approval before submission.

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