Efficacy of a bivalent HPV 16/18 vaccine against anal HPV 16/18 infection among young women: a nested analysis within the Costa Rica Vaccine Trial

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Summary

Background **Anal cancer remains rare (incidence of about 1·5 per 100 000 women yearly), but rates are increasing in many countries. Human papillomavirus (HPV) 16 and 18 infections cause most cases of anal cancer. We assessed efficacy of an AS04-adjuvanted HPV 16 and HPV 18 vaccine against anal infection with HPV 16, HPV 18, or both (HPV 16/18).**

Methods **Women from Costa Rica were registered between June 28, 2004, and Dec 21, 2005, in a randomised double-blind controlled trial that was designed to assess vaccine efficacy against persistent cervical HPV 16/18 infections and associated precancerous lesions. Eligible women were residents of Guanacaste and selected areas of Puntarenas, Costa Rica, age 18–25 years, in good general health, willing to provide informed consent, and were not pregnant or breastfeeding. Participants were randomly assigned (1:1) to receive an HPV vaccine (Cervarix, GlaxoSmithKline, Rixensart, Belgium) or a control hepatitis A vaccine (modified preparation of Havrix, GlaxoSmithKline, Rixensart, Belgium). Vaccines were administered in three 0·5 mL doses at enrolment, 1 month, and 6 months. Women, selected at the final blinded study visit 4 years after vaccination, provided anal specimens for assessment of vaccine efficacy against anal HPV 16/18 infection. Prevalence of anal HPV 16/18 infections, reported as vaccine efficacy, was the primary endpoint of the study described here. Vaccine efficacy against cervical HPV 16/18 infection in the same women at the 4-year visit was used as a comparator. Analyses were done in a restricted cohort of women who were negative for both cervical HPV 16 and HPV 18 DNA and who were HPV 16 and HPV 18 seronegative before enrolment (HPV naive), and also in the full cohort of women who provided an anal specimen. Investigators were masked to group assignment. This study is registered at ClinicalTrials.gov, number NCT00128661.**

Findings **All women who attended the final blinded study visit and consented to anal specimen collection were included in the analysis (4210 of 6352 eligible women). In the full cohort, vaccine efficacy against prevalent HPV 16/18 infection measured one-time, 4 years post vaccination was lower at the anus (62·0%, 95% CI 47·1–73·1) compared with the cervix (76·4%, 67·0–83·5; p for interaction by anatomical site 0·031). In the restricted cohort, vaccine efficacy against anal HPV 16/18 infection was 83·6% (66·7–92·8), which was similar to vaccine efficacy against cervical HPV 16/18 infection (87·9%, 77·4–94·0). Safety issues were not addressed in the current analysis. Additional safety data will be published later in a separate article.**

Interpretation **The AS04-adjuvanted vaccine affords strong protection against anal HPV infection, particularly among women more likely to be HPV naive at enrolment.**

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Introduction

Anal cancer remains rare, with an annual age-standardised incidence in the general population of about 1·5 per 100 000 for women;[1] but rates have roughly doubled in recent decades in many countries, including the USA and several European nations.[2–5] The absolute burden of anal cancer is higher for women than men,[3–5] yet, anal cancer disproportionately affects HIV-positive individuals and men who have sex with men, even if they are HIV-negative.[6,7]

Human papillomavirus (HPV) causes most anal cancers, with an estimated 75–80% of HPV-associated anal cancers caused by HPV types 16 or 18.[8,9] For other HPV-associated extracervical cancers, including cancers of the oropharynx, vagina, vulva, and penis,[9] variable proportions are caused by HPV infection but, when HPV is present, HPV 16 is the predominant type implicated (75–95% of the HPV-associated cancers).[9]

Two vaccines prevent infection with HPV 16 and HPV 18: the bivalent HPV 16 and HPV 18 vaccine (Cervarix, GlaxoSmithKline Biologicals, Rixensart, Belgium)[10] and the quadrivalent HPV 6, 11, 16, and 18 vaccine (Gardasil, Merck, Whitehouse Station, NJ, USA).[11] In women, vaccine efficacy has been shown

against cervical precancer (both vaccines)[10,11] and vaginal and vulvar HPV infections and related diseases (quadrivalent only).[12] For men, vaccine efficacy has been shown against penile, perianal, perineal HPV infections as a combined endpoint (quadrivalent only).[13] To our knowledge, direct evidence for efficacy against anal HPV infection has only been shown in one unpublished trial[14] of the quadrivalent vaccine in about 600 men who have sex with men. The bivalent vaccine has not been assessed at extracervical sites.

We assessed the efficacy of the bivalent HPV vaccine to decrease anal HPV infection using data nested in a community-based randomised trial of cervical vaccine efficacy in young adult women.

Methods

Patients

Women included in this study were participants in a double-blind, randomised clinical trial initially designed to assess the efficacy of a bivalent HPV vaccine against persistent type-specific infection with HPV 16, HPV 18, or both (from here on referred to as HPV 16/18) and associated precancerous lesions at the cervix.[15,16] The study enrolled women residing in Guanacaste and selected areas of Puntarenas, Costa Rica, identified via a census, between June 28, 2004, and Dec 21, 2005. Main eligibility requirements were: age 18–25 years, planned residence in the area of Guanacaste for the 6 months following enrolment, in good general health, neither pregnant nor breastfeeding, and willing to provide written informed consent. Women were excluded if they had pre-existing medical disorders that needed chronic treatment or caused immunosuppression, had a history of hepatitis A or previous vaccination against it, or were unwilling to use contraception during the vaccination period. 7466 women were enrolled; they represented 59% of 12 624 potentially eligible women and 31% of all 24 467 women screened from the census.[15] The trial was approved by the human subjects review committees of the US National Cancer Institute and by the Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA) in Costa Rica. All women gave written informed consent.

Randomisation and masking

Patients were randomly assigned (1:1) to receive an AS04-adjuvanted HPV vaccine (Cervarix) or a control hepatitis A vaccine (a modified preparation of Havrix, GlaxoSmithKline Biologicals, Rixensart, Belgium). HPV and control vaccines were formulated in three doses of 0·5 mL with identical packaging. Both vaccines were assigned random vaccine identification numbers at the time of labelling by the manufacturer. These numbers were randomised by the study data management centre (Information Management Services, Silver Spring, MD) under contract with the National Cancer Institute (NCI) with a standard SAS (SAS 9.2 TS2M3) programme. First dose syringes for both vaccines were combined, sorted in numerical order, and delivered in sequentially numbered boxes to the study site in Costa Rica. Masked study personnel randomly assigned every eligible participant to the next available sequential vaccine identification number. The syringes for the second and third doses were selected on the basis of linkage of the vaccine ID to the first dose so as to maintain the same material type for every participant.

All field workers were masked to group assignment (ie, interviewers, clinicians, colposcopists, pathologists, technicians, outreach workers, drivers, quality control); as well as investigators from the USA and Costa Rica, participants, and medical monitors. Codes were kept at the study's data management centre (IMS, under contract with NCI) and GlaxoSmithKline under controlled and secured access.

Unmasking of individual participants, at the request of the data safety monitoring board, the institutional review boards, the GlaxoSmithKline Global Clinical Safety Department as part of reporting requirements to the US Food and Drug Administration (FDA; eg, for rapid reporting of an unexpected serious adverse event associated with vaccination) was allowed, with previous approval by the NCI medical monitors. This individual unmasking was done in a way that assured that the overall study masking was maintained and all unmaskings were documented appropriately.

Procedures

At the enrolment visit (after risk-factor interview), a pelvic examination was done on sexually experienced women, exfoliated cervical cells were kept in PreservCyt medium (Cytyc Corp, now Hologic, Marlborough, MA, USA) for Thinprep (Cytyc Corp) cytological assessment and HPV DNA testing, and blood samples were obtained for HPV 16 and HPV 18 serological tests. Next, women were randomly assigned to receive either the HPV vaccine or a control hepatitis A vaccine. The protocol called for a dose of vaccine at all three study visits: at enrolment, 1 month after the initial dose (allowable range 21–120 days after enrolment), and 6 months after the initial dose (allowable range 121–300 days). Women not attending their visits in the allowable range for the second dose remained eligible for the final dose; women who missed the window for the final dose did not receive that dose.[15]

At annual follow-up visits, clinicians obtained from sexually active women exfoliated cervical cells (same method as above) for cytological assessment and HPV DNA testing. Women with low-grade cytological abnormalities were assessed every 6 months until three consecutive normal cytological results, at which point they were followed up yearly. Women with cervical high-grade disease or persistent low-grade abnormalities were referred to cervical colposcopy for assessment and treatment if needed.

Sampling of the anus was introduced at the 4-year study visit, the final blinded study visit of the trial. At this study visit, women were given a questionnaire that included questions on anal sexual behaviours.

The anal specimen was obtained before the pelvic examination in sexually active women (defined by vaginal intercourse) by insertion of a dry swab of 3–4 cm into the anal canal, one rotation, and then removal of the swab while rotation continued using gentle pressure against the wall of the anal canal. The swab was placed in 1 mL of PreservCyt medium and frozen immediately in liquid nitrogen. Although the predictive value of one-time detection of anal HPV 16/18 for the development of anal precancer or cancer is probably very low, and there are no standard clinical recommendations for follow-up of such cases, a subset of women with anal HPV 16/18 infection will be monitored during the long-term follow-up phase of this trial. Most women will be followed up to 10 years after initial vaccination. As part of this effort, long-term type-specific anal HPV persistence and related disease will be monitored, and clinical management will be provided where necessary.

Anal and cervical samples were tested for HPV DNA with the SPF10 PCR primer system and a DNA enzyme immunoassay detection of amplimers (DEIA; DDL Diagnostic Laboratory, Voorburg, Netherlands); if positive, genotyping was done with the line-probe assay (LiPA25; SPF10PCR/LiPA25 HPV genotyping assay system, version 1, Labo Bio-medical Products, Rijswijk, Netherlands).[17] LiPA25 detects 25 HPV genotypes, including carcinogenic (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 or 73) and non-carcinogenic (6, 11, 34, 40, 42, 43, 44, 53, 54, 66, 70, and 74) types. To ensure that HPV 16 and HPV 18 infections were not missed, all positive specimens on SPF10 PCR/DEIA that were negative for HPV 16 or HPV 18 by LiPA25 were also tested with type-specific primers for HPV 16 and HPV 18.[18,19]

Serum samples obtained at enrolment were used to identify HPV 16 and HPV 18 serological status with a VLP-based direct enzyme-linked immunosorbent assay (ELISA), a standard test that measures polyclonal antibodies (GlaxoSmithKline Biologicals, Rixensart, Belgium), as described previously.[20,21] Antibody results were dichotomised with standard cutoff points calculated from antibody-titre values 3 SDs above the geometric mean titres taken from a group of HPV-negative individuals.[20] Cutpoints were an optical density of at least 8 ELISA units (EU)/mL for anti-HPV 16 and at least 7 EU/mL for anti-HPV 18.[20,21]

Statistical analysis

Characteristics between women who accepted and declined the anal specimen collection were compared with the χ^2 test for categorical variables. In women who accepted anal specimen collection, patient characteristics from both the enrolment and the 4-year post-vaccination visits were compared between women in the HPV and control groups. Median follow-up time from enrolment was calculated in months overall and compared by group with the Kruskal-Wallis test.

Prevalence of anal HPV 16 or 18 infections roughly 4 years after vaccination was the primary endpoint (defined as detection of either HPV 16 or HPV 18, or both, at the 4-year study visit); cervical HPV 16 or 18 infections in the same women at this timepoint were assessed for comparison purposes. Prevalence findings were then converted and reported as vaccine efficacy.

The full analysis cohort included all women who had given anal specimens and had HPV results available

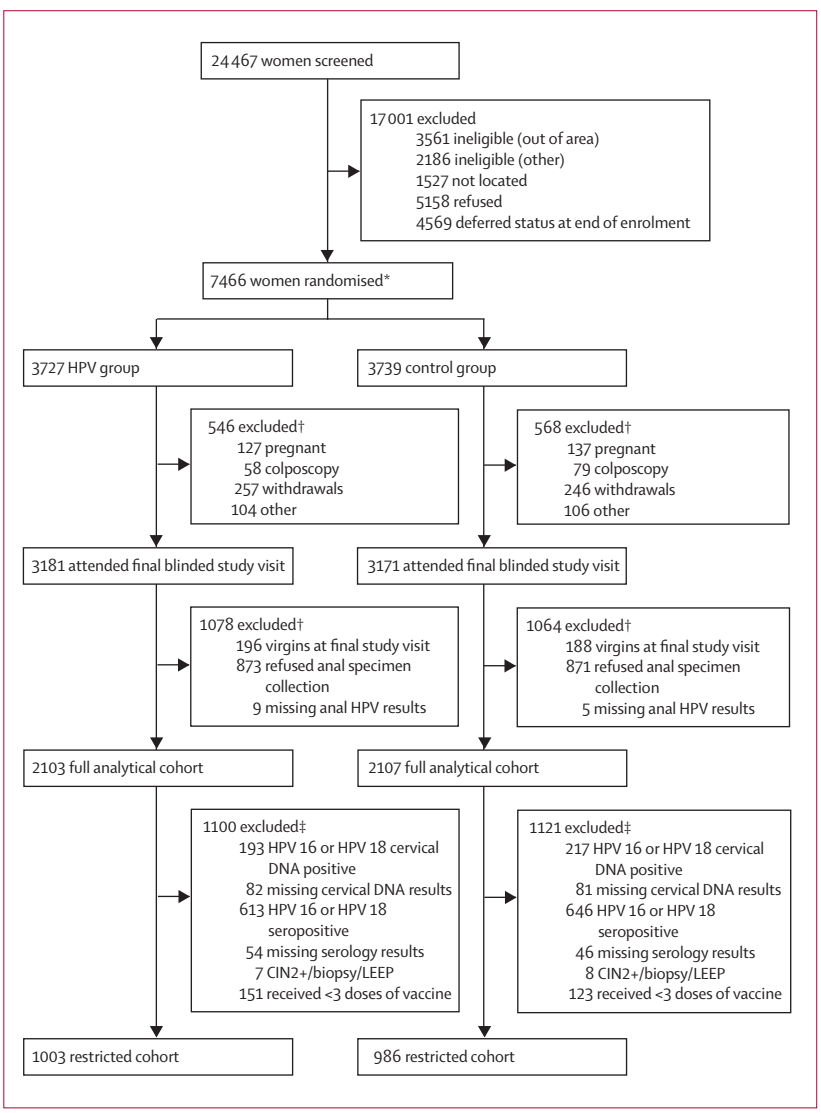

Figure: **Trial profile**

HPV=human papillomavirus. CIN2+=cervical intraepithelial neoplasia grade 2 or higher. LEEP=loop electrosurgical excision procedure. *Four women received discordant vaccines (one woman was enrolled twice and received three doses of each vaccine and three women received two doses of one vaccine and one dose of the other vaccine). For the aim of this analysis, the women were assigned to the group for which the first dose was given. †Data obtained at the 4-year study visit at which anal specimens were obtained were used to create the full analytical cohort. ‡Data obtained at enrolment (cervical HPV DNA and serological status) and throughout the vaccination phase (CIN2+, biopsy, and LEEP, and fewer than three doses) were used to exclude women from the restricted cohort.

(full cohort); thus, in this cohort, no exclusions were based on HPV DNA positivity, HPV serostatus, or number of vaccine doses received. The restricted cohort included only women with no evidence of prevalent cervical HPV 16 and HPV 18 infection or HPV 16 and HPV 18 antibodies before vaccination (HPV naive group), who received three doses of the HPV or control vaccine. We intended to remove women from this cohort who might have had past or prevalent anal HPV infection[22] by restricting on the basis of cervical infections that are correlated with concomitant anal infections (ie, in our population, type-specific HPV 16 agreement at the anus and the cervix measured at the 4-year study visit was 31% (47 of 154) positive agreement and κ was 0·44). This was necessary as a prevaccination anal specimen was not available to allow exclusion on the basis of direct detection of anal HPV. Women were further excluded from the restricted cohort if they met any of the following criteria: (1) missing results or positive results for cervical HPV 16 or HPV 18 DNA or HPV 16 or HPV 18 seropositivity at enrolment, (2) biopsy samples for possible cervical intraepithelial neoplasia or treated by loop electrosurgical excision procedure (LEEP), after a positive screening test during the vaccination phase (eg, until the 6-month study visit, which in practice could occur 4–10 months after enrolment), or (3) recipients of fewer than three doses of either vaccine.

	HPV (n=2103)	Control (n=2107)	p value
Age at entry (years)*			0·3
18–19	595 (28%)	639 (30%)	..
20–21	516 (25%)	496 (24%)	..
22–23	511 (24%)	524 (25%)	..
24–25	481 (23%)	448 (21%)	..
Cervical HPV 16 and HPV 18 DNA status at enrolment†			0·2
Negative	1646 (90%)	1615 (88%)	..
Positive‡	193 (10%)	217 (12%)	..
Serological HPV 16 and HPV 18 status at enrolment§			0·1
Negative	1246 (61%)	1210 (59%)	..
Positive‡	791 (39%)	837 (41%)	..
Enrolment cervical cytology†			0·6
Inadequate	8 (<1%)	10 (<1%)	..
Normal	1544 (84%)	1557 (85%)	..
HPV-positive ASCUS or LSIL	246 (13%)	233 (13%)	..
HSIL	41 (2%)	32 (2%)	..
Time since sexual debut (measured at enrolment visit)¶			0·95
Virgin at enrolment	264 (13%)	275 (13%)	..
<2 years	206 (10%)	211 (10%)	..
2–3 years	435 (21%)	430 (20%)	..
≥4 years	1198 (57%)	1191 (57%)	..
Anal sex‖			0·2
No	1613 (77%)	1655 (79%)	..
Yes	490 (23%)	452 (21%)	..

All data are n (%). Full analysis cohort included all women who accepted anal specimen collection. HPV=human papillomavirus. ASCUS=atypical cells of undetermined significance. LSIL=low-grade squamous intraepithelial lesion. HSIL=high-grade squamous intraepithelial lesion. *Two women enrolled at age 17 years are included in the 18–19 year age group. †No cervical specimen was obtained for HPV testing or cervical cytology in virgins (n=264 in HPV group and n=275 in control group). ‡Indicates positive for either or both HPV 16 and HPV 18 at enrolment. §66 women in the HPV group and 60 women in the control group had missing serological results. ¶The few (n<5) "unknown" responses were collapsed into the category of "4 or more years". ‖The few (n<5) "refused" and "don't know" responses were collapsed into the "no" category.

Table 1: **Patient characteristics by vaccine group for the full analytical cohort**

For every group, the prevalences of anal and cervical HPV 16/18 infections combined, and measured separately, 4 years post-vaccination were shown as the number of infected women per 100 women vaccinated (stratified by HPV *vs* control vaccine); we estimated asymptotic CIs (95% CIs) except when cells had less than five events, in which case we reported exact confidence intervals. The complement of the ratios of the prevalence for the HPV and control groups provided the vaccine efficacy estimates. In this patient-level analysis, every woman could only contribute once to the numerator and denominator, even if several HPV types were detected. We calculated exact CIs[23] for vaccine efficacy on the basis of the binomial distribution of the number of events in the HPV group among the total number of events in the HPV and control groups.[24] We calculated and compared anal and cervical estimates of vaccine efficacy by including a variable for the interaction between group and anatomical site in a generalised estimating equation (GEE) model[25] and assessing whether the β coefficient for the interaction variable varied significantly from 0.

In our prespecified plan, the main objective of our analysis was to assess vaccine efficacy against anal HPV 16/18 infection 4 years after enrolment and administration of the first vaccine dose (regardless of vaccine type); cervical vaccine efficacy in the exact same cohorts was estimated as a comparator. Because of evidence for cross-protection in cervical vaccine efficacy studies,[10,11] we also assessed anal and cervical vaccine efficacy against a prespecified composite endpoint of HPV 31/33/45, and then individually by type. In the restricted cohort, we excluded women who were DNA positive for HPV 31, 33, or 45 at enrolment from the respective analysis; we did not make serological restrictions based on HPV types 31, 33, or 45 because these assays were not done. Anal and cervical vaccine efficacy against all other carcinogenic types (after removing HPV 16, 18, 31, 33, and 45) was also assessed in the full cohort.

HPV 16/18 vaccine efficacy was estimated by self-reported anal sex (yes or no) assessed by questionnaire at the study visit when the anal specimen was obtained, in the full and restricted cohorts. At the time of this analysis, fieldwork was ongoing and individual information remained blinded. Thus, analyses were done by an external group, Information Management Systems (by Sabrina Chen; Rockville, MD), under the direction of the investigators who remained masked to individual random assignments. SAS 9.2 TS2M3 was used for analysis and a p value of less than 0·05 was considered significant.

This study is registered at ClinicalTrials.gov, number NCT00128661.

Role of the funding source

The NCI and Costa Rica investigators were responsible for the study design, data collection, data management, data analysis, interpretation and preparation of the report. The corresponding author had access to all summary level data. The NCI and Costa Rica investigators had final responsibility for the decision to submit for publication. GlaxoSmithKline had the right to review and comment on the report.

Results

Of the 7466 women randomly assigned to HPV or control vaccines, 6352 attended the 4-year study visit (figure). 384 of women were virgins and therefore not eligible for anal specimen collection. Another 1744 women refused anal specimen collection. After exclusion of 14 missing anal HPV results because of inadequate specimen volume, the full analytical cohort consisted of 4210 women. Median follow-up time was 48·8 months (4·1 years) and was similar between groups (HPV 48·9 months [range 39·5–74·5] and control 48·8 months [38·7–73·4]; p=0·9). The restricted cohort of women who were negative at enrolment for cervical HPV 16 and HPV 18 DNA and negative by serology included 1989 women.

The proportion of women who accepted anal specimen collection was similar in both groups (71% for both HPV [2112 of 2985] and control [2112 of 2983] groups). Patient characteristics, despite attrition in the number of women over time, remain roughly similar in the HPV vaccine and control groups at 4 years to that seen in the enrolment groups (data not shown). Women who provided an anal specimen were older (1964 [47%] of 4210 women were in the older age category at enrolment [22–25 years]) than those who refused to give a specimen (695 [40%] of 1744 in the older age category at enrolment; p<0·0001), more likely to have cervical HPV 16 or 18 positivity at enrolment (10% [410 of 4210] *vs* 5% [90 of 1744], p<0·0001), more likely to have four or more lifetime sexual partners (38% [1598 of 4210] *vs*

	Number of women	Number of HPV 16/18 infections	Prevalence of HPV 16/18 (95%CI)	HPV vaccine efficacy (95%CI)
Full cohort*				
Anus				62·0% (47·1–73·1)†
HPV	2103	47	2·2% (1·7–2·9)	..
Control	2107	124	5·9% (4·9–7·0)	..
Cervix				76·4% (67·0–83·5)†
HPV	2103	40	1·9% (1·4–2·6)	..
Control	2107	170	8·1% (7·0–9·3)	..
Restricted cohort‡				
Anus				83·6% (66·7–92·8)§
HPV	1003	8	0·8% (0·4–1·5)	..
Control	986	48	4·9% (3·7–6·3)	..
Cervix				87·9% (77·4–94·0)§
HPV	1003	10	1·0% (0·5–1·8)	..
Control	986	81	8·2% (6·6–10·1)	..

HPV=human papillomavirus. *Full analysis cohort included all women who accepted anal specimen collection. †p value for difference in vaccine efficacy by anatomical site was 0·031. ‡Restricted cohort included women from the full cohort with no evidence of prevalent cervical HPV 16 and HPV 18 infection or HPV 16 and HPV 18 antibodies before vaccination, who received three doses of the HPV or control vaccines. §p value for difference in vaccine efficacy by anatomical site was 0·55.

Table 2: **Estimated vaccine efficacy against anal and cervical HPV 16/18 infections**

	Number of women	Number of HPV 31/33/45 infection	Prevalence of HPV 31/33/45 (95%CI)	HPV 31/33/45 vaccine efficacy (95%CI)
Full cohort*				
Anus				49·4% (30·3–63·6)†
HPV	2103	55	2·6% (2·0–3·4)	..
Control	2107	109	5·2% (4·3–6·2)	..
Cervix				45·2% (27·7–58·7)†
HPV	2103	76	3·6% (2·9–4·5)	..
Control	2107	139	6·6% (5·6–7·7)	..
Restricted cohort‡				
Anus				61·8% (42·8–75·0)§
HPV	1629	31	1·9% (1·3–2·7)	..
Control	1684	84	5·0% (4·0–6·1)	..
Cervix				51·3% (31·9–65·5)§
HPV	1629	49	3·0% (2·3–3·9)	..
Control	1684	104	6·2% (5·1–7·4)	..

HPV=human papillomavirus. *Full analysis cohort included all women who accepted anal specimen collection. †p value for difference in vaccine efficacy by anatomical site was 0·65. ‡Restricted cohort included women from the full cohort with no evidence of prevalent cervical HPV 31, 33, or 45 infections before vaccination, and who received three doses of the HPV or control vaccine. §p value for difference in vaccine efficacy by anatomical site was 0·28.

Table 3: **Estimated vaccine efficacy against anal and cervical infection with HPV 31, 33, or 45**

	Number of women	Number of HPV 16/18 Infections	Prevalence of HPV 16/18 (95%CI)	HPV 16/18 vaccine efficacy (95%CI)
Full cohort*				
Anus				
No anal sex				55·3% (33·5–70·4)†
HPV	1613	34	2·1% (1·5–2·9)	..
Control	1655	78	4·7% (3·8–5·8)	..
Anal sex				73·9% (52·7–86·4)†
HPV	490	13	2·7% (1·5–4·4)	..
Control	452	46	10·2% (7·6–13·2)	..
Cervix				
No anal sex				76·2% (64·7–84·3)‡
HPV	1613	29	1·8% (1·2–2·5)	..
Control	1655	125	7·6% (6·4–8·9)	..
Anal sex				77·5% (57·4–88·8)‡
HPV	490	11	2·2% (1·2–3·9)	..
Control	452	45	10·0% (7·4–13·0)	..
Restricted cohort§				
Anus				
No anal sex				85·0% (63·8–94·8)¶
HPV	808	5	0·6% (0·2–1·4)	
Control	799	33	4·1% (2·9–5·7)	
Anal sex				80·8% (38·8–95·6)¶
HPV	195	3	1·5% (0·4–4·1)	
Control	187	15	8·0% (4·7–12·6)	
Cervix				
No anal sex				88·3% (75·5–95·1)‖
HPV	808	7	0·9% (0·4–1·7)	
Control	799	59	7·4% (5·7–9·4)	
Anal sex				86·9% (60·3–96·9)‖
HPV	195	3	1·5% (0·4–4·1)	
Control	187	22	11·8% (7·7–17·0)	

*Full analysis cohort included all women who accepted anal specimen collection. †p value for difference in vaccine efficacy at anus by anal sex status was 0·13. ‡p value for difference in vaccine efficacy at cervix by anal sex status was 0·89. §Restricted cohort included women from the full cohort with no evidence of prevalent cervical HPV 16 or HPV 18 infection or HPV 16 or HPV 18 antibodies before vaccination, who received three doses of the HPV or control vaccine. ¶p value for difference in vaccine efficacy at anus by anal sex status was 0·76. ‖p value for difference in vaccine efficacy at cervix by anal sex status was 0·88.

Table 4: **Estimated vaccine efficacy against anal and cervical infections with HPV 16/18 by self-reported anal-sex status in the full and restricted cohorts**

See Online for webappendix

31% [546 of 1744], p<0·0001), and more likely to report anal sex (22% [942 of 4210] *vs* 8% [143 of 1744], p<0·0001) than women who declined.

Characteristics after randomisation were well balanced between groups (table 1) in the full cohort. In the full cohort, vaccine efficacy against anal HPV 16/18 infection detected 4 years after vaccination was 62·0% (95% CI 47·1–73·1) and the corresponding cervical vaccine efficacy was 76·4% (67·0–83·5); p for interaction by anatomical site was 0·031; table 2). Vaccine efficacy against anal HPV 16 was 68·2% (51·4–79·7; 27 events in the HPV group *vs* 85 events in the control group) and against anal HPV 18 was 55·5% (25·2–74·2; 20 events in the HPV group and 45 events in the control group); vaccine efficacy against cervical HPV 16 was 75·8% (63·8–84·2; 28 events in the HPV group *vs* 116 in the control group) and against cervical HPV 18 infection was 78·6% (62·0–88·7; 13 events in the HPV group *vs* 61 events in the control group; p for interaction by anatomical site was 0·3 for HPV 16 and 0·05 for HPV 18).

In the restricted cohort, patients' characteristics were well balanced too (data not shown). Vaccine efficacy against anal HPV 16/18 infection (83·6%) detected 4 years after administration of the first dose of vaccine was similar to the cervical HPV 16/18 vaccine efficacy (87·9%) in the same women from specimens obtained at the same timepoint (table 2).

In the full cohort, similar cross-protection against a composite endpoint of infection with HPV 31/33/45 was shown at the anus and the cervix (table 3). Individually, vaccine efficacy was present against HPV 31 and 45 but not 33 (webappendix p 1). The results for vaccine efficacy against heterologous HPV types in the full cohort were similar to those in the restricted cohort (table 2 and webappendix p 1). No vaccine efficacy was noted for all other carcinogenic HPV types (after exclusion of types 16, 18, 31, 33, and 45; for the full cohort, anal vaccine efficacy was –3·2% [95% CI –18·6 to 10·1], 405 events in the HPV group *vs* 393 anal events in the control group and cervical vaccine efficacy was 3·9% [–9·4 to 15·6], 445 events in the HPV group *vs* 464 cervical events in the control group).

At the final study visit, women in the full cohort who reported anal sex had an anal vaccine efficacy of 73·9% (95% CI 52·7–86·4) whereas those who did not report anal sex had an anal vaccine efficacy of 55·3% (33·5–70·4; p for interaction by anal sex status 0·13); by contrast, cervical vaccine efficacy was similar between reported anal sex statuses (table 4). In the restricted cohort, anal and cervical vaccine efficacy estimates were similarly high, regardless of anal sex status (table 4).

When the FDA licensed the HPV vaccine, safety data from our trial and others were reviewed and the vaccine was deemed safe. The main safety issue to arise—the effect of vaccination on pregnancies and miscarriages—has been addressed in a previous publication.[26] Additional safety data will be published in a separate article that reports vaccine efficacy at the cervix, the primary and secondary objectives of our trial.

Discussion

A randomised analysis of data from our community-based HPV vaccine trial in Costa Rica shows that the bivalent HPV vaccine is efficacious against prevalent anal HPV 16/18 infections in young women measured 4 years after vaccination. We also show, to our knowledge for the first time, evidence of cross-protection against a composite endpoint of HPV types 31/33/45 at an extragenital site; providing confirmation that the protection afforded by the bivalent HPV vaccine goes

beyond the HPV types included in the vaccine formulation.

Our estimate of vaccine efficacy for anal HPV 16/18 infection for the bivalent vaccine in our full cohort is similar to the only other study assessing the efficacy of an HPV vaccine against anal infection and related disease.[14] In that study, vaccine efficacy of the quadrivalent vaccine against anal HPV 16/18 infection and related lesions was 55% in 598 HIV-negative men who have sex with men (115 total events) in their full analytical cohort (age 16–26 years, median follow-up of 32 months).[14]

Vaccine efficacy for anal HPV infection in women who reported anal sex, and who were therefore at increased risk for anal cancer,[27] seemed higher than that in women who reported no anal sex in the full cohort; a finding not replicated in the restricted cohort. One possibility might be that a greater proportion of the anal infections detected in women who did not have anal sex might be superficial virions shed from genital sites for which the vaccine would not be expected to protect. Future studies that assess anal vaccine efficacy in individuals who have anal sex would benefit from querying the timing of anal specimen collection relative to timing of most recent anal sex.

The main limitation of our analysis is that only one anal specimen was obtained, 4 years after vaccination. We were therefore unable to assess anal HPV infection before vaccination, or use HPV incidence or persistence as our endpoint instead of prevalence. Estimated vaccine efficacy is reduced by inclusion of women with prevalent infection at the time of vaccination, not protected by this prophylactic vaccine.[16] Persistent infection is a preferred trial endpoint over one-time detection because it reduces measurement error, is likely associated with higher absolute risk of anal cancer, and is therefore a better proxy for cancer prevention. Thus, evaluation of vaccine efficacy against persistent anal HPV infection and associated lesions is necessary in women. Further, showing that the protection lasts beyond the 4 years assessed in this study will be important to ensure women are protected during the ages of higher exposure. Moreover, although some under-reporting of stigmatised behaviours such as anal sex is expected, we did not predict differential reporting by vaccination status that would bias our efficacy estimates.

Our study is likely to have internal validity because: it is randomised, most women agreed to specimen collection, and the patient characteristics of those women who refused to have specimens collected did not differ by arm. While external validity of our findings could be questioned, it is important to note that our trial is the only pre-licensure study that enrolled participants from a defined catchment area based on census information, thereby maximising the likelihood of external validity.

Panel: Research in context

Systematic review

We searched the literature using several search engines including PubMed using key words related to anal HPV infection and the prophylactic HPV vaccines and found no published trials on vaccine efficacy against anal HPV infection in women. Yet, data from epidemiological studies suggest an important role of HPV in the aetiology of anal cancers and their precursors. Current HPV vaccines protect against mucosal HPV infections at the cervix and it therefore made sense to assess whether HPV infections would protect against infection at another mucosal site in which HPV can cause cancer—the anus. Furthermore, an unpublished study[14] has reported a vaccine efficacy of around 50% against anal infection and related diseases with use of a quadrivalent HPV vaccine in men.

Interpretation

Since our data show a reduction of anal HPV infection rates in vaccinated women, it suggests that, in the future, women who receive the prophylactic HPV vaccines before exposure to the virus will possibly have less anal cancer.

HPV vaccines have great potential for prevention of a large proportion of HPV-associated cancers at the anus and other anatomical sites (panel), assuming adequate duration of protection. In women, published evidence exists for vaccine efficacy against HPV 16/18 infections at the cervix, vagina, vulva,[10–12] and now anus, and for men, protection has been shown at genital and anal anatomical sites.[13] While vaccine efficacy against oral HPV infection has not been shown, vaccination might also prevent some HPV-associated oropharyngeal cancers. The implications that HPV causes extracervical cancers, and that the vaccine protects against the infections that cause these cancers, differs between countries with and without effective screening programmes for cervical cancer. Countries without screening for cervical cancer have high rates of cervical cancer and the absolute burden of HPV-associated cancers will remain many times higher for the cervix than for the combined non-cervical sites, implying that limited resources for vaccination should be focused on women and not men. However, high-resource countries with screening for cervical cancer typically have dramatically diminished rates of cervical cancer. The use of HPV vaccines in men might be affordable and justifiable because the number of HPV-associated cancers might in the future be greater in men, since rates of anal and oropharyngeal cancers are increasing, and oropharyngeal cancer is predominantly diagnosed in men.[28]

Our findings suggest that the bivalent HPV vaccine protects against a proportion of anal HPV 16 and HPV 18 infections. Findings presented here and in the

literature suggest that the incidence of HPV-associated cancers at several anatomical sites will be decreased in women who receive the prophylactic HPV vaccines before exposure to the virus.

Contributors

ARK, PG, HAK, SW, AH, RH, DS, and MS designed the analysis. SC did all statistical programming under the direction of ARK. ARK, PG, SW, ACR, AH, RH, CP, DS, MS, and SJ were responsible for data collection. WQ, L-JvD, and LS were responsible for all HPV-related test results. ARK, PG, HAK, SW, AH, RH, DS, and MS analysed the data. ARK, PG, HAK, SW, ACR, AH, RH, CP, DS, MS, DRL, and JTS interpreted the data. ARK wrote the report. ARK, PG, HAK, SW, ACR, AH, RH, DS, MS, DRL, JTS, and SC critically reviewed all material for important intellectual content. ARK is the guarantor of all material contained herein.

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Conflicts of interest

JTS and DRL report that they 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. They are entitled to limited royalties as specified by federal law. The other authors declare that they have no conflicts of interest.

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