Cross-protection of the Bivalent Human Papillomavirus (HPV) Vaccine Against Variants of Genetically Related High-Risk HPV Infections

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Background. Results from the Costa Rica Vaccine Trial (CVT) demonstrated partial cross-protection by the bivalent human papillomavirus (HPV) vaccine, which targets HPV-16 and HPV-18, against HPV-31, -33, and -45 infection and an increased incidence of HPV-51 infection.

Methods. A study nested within the CVT intention-to-treat cohort was designed to assess high-risk HPV variant lineage–specific vaccine efficacy (VE). The 2 main end points were (1) long-term incident infections persisting for ≥2 years and/or progression to high-grade squamous intraepithelial lesions (ie, cervical intraepithelial neoplasia grade 2/3 [CIN 2/3]) and (2) incident transient infections lasting for <2 years. For efficiency, incident infections due to HPV-16, -18, -31, -33, -35, -45, and -51 resulting in persistent infection and/or CIN 2/3 were matched (ratio, 1:2) to the more-frequent transient viral infections, by HPV type. Variant lineages were determined by sequencing the upstream regulatory region and/or E6 region.

Results. VEs against persistent or transient infections with HPV-16, -18, -33, -35, -45, and -51 did not differ significantly by variant lineage. As the possible exception, VEs against persistent infection and/or CIN 2/3 due to HPV-31 A/B and HPV-31C variants were −7.1% (95% confidence interval [CI], −33.9% to 0%) and 86.4% (95% CI, 65.1%–97.1%), respectively (*P* = .02 for test of equal VE). No difference in VE was observed by variant among transient HPV-31 infections (*P* = .68).

Conclusions. Overall, sequence variation at the variant level does not appear to explain partial cross-protection by the bivalent HPV vaccine.

Keywords. HPV vaccine; cross-protection; HPV variants.

Persistent infection with high-risk human papillomaviruses (HPVs) causes cervical cancer, a very common female malignancy worldwide [1]. Although >200 HPV genotypes have been characterized, cervical cancer is associated with a limited set of 12 related types from 5 *Alphapapillomavirus* species (alpha-5, -6, -7, -9, and -11) [2–4]. HPV-16 and HPV-18 cause approximately 70% of cervical cancers [5] and are targeted by the virus-like particle–based prophylactic HPV vaccines Gardasil and Gardasil9 (Merck, Whitehouse Station, New Jersey) and Cervarix (GlaxoSmithKline Biologicals, Rixensart, Belgium). All are highly effective against HPV-16/18 persistence [6] and cervical

intraepithelial neoplasia grade 2/3 (CIN 2/3) [7–9]. Development of neutralizing antibodies against HPV-16/18 is correlated with reported cross-protection against the phylogenetically HPV-16–related types 31 and 33 and the HPV-18–related type 45 [10, 11]. Partial cross-protection has been demonstrated in studies using either the bivalent or quadrivalent vaccine [12], while variable and/or increased rates of infections have been observed for HPV-51, -52, and -58 [12–14].

Variant lineages are classified as viral genomes of a known HPV type with <10% sequence variability [15]. Specific variant lineages show epidemiologic patterns in their distribution and associations with CIN 2/3 and cancer [15, 16]. In addition, HPV variants are associated with hosts of common geographic ancestry and are even evident with population movements; thus, not all geographic regions harbor similar variants (or at similar frequencies) [15, 17–19]. Whether viral genomic polymorphisms associated with variant lineages alter infection rates upon vaccination has not been thoroughly evaluated, but this is of interest because variants exhibit differences in natural history and disease risks [15, 17, 20–22]. Although vaccine protection against HPV-16/18 is uniformly high, cross-protection against

genetically related high-risk HPV types is variable and could be due to differential protection against variant lineages [15].

This study was designed to assess the impact of HPV-16/18 vaccination on incident infection(s) at the viral variant level. This study evaluated the relative vaccine efficacy (VE) by variant lineage in samples obtained from young women participating in the Costa Rica Vaccine Trial (CVT), a National Cancer Institute (NCI)–sponsored community-based, double-blind, randomized clinical trial of Cervarix in Guanacaste, Costa Rica. The present study examined the hypothesis that viral variants circulating in Guanacaste were differentially affected by HPV vaccination.

MATERIALS AND METHODS

The study of viral variants was a nested analysis within the previously reported CVT (clinical trials registration NCT00128661). Written informed consent was obtained from all participants in CVT. Institutional review board approval was obtained for the informed consent forms at both the NCI and in Costa Rica.

CVT Study Design

The study design and main outcomes have been published previously [6, 23, 24]. Briefly, the study involved female residents living in Guanacaste and Puntarenas, Costa Rica, with community-based census recruitment occurring during 2004–2005. A total of 7466 women aged 18–25 years were randomly assigned (ratio, 1:1) to receive the HPV-16/18 vaccine, Cervarix, or control (hepatitis A vaccine; Havrix, GSK Biologicals).

Prior to vaccination, all women completed a study survey and had a clinical visit; specimens collected prior to randomization were classified as having been collected at enrollment. Vaccination with 3 doses occurred over 6 months in the majority of women in the CVT; 71.5% of participants received doses within the prespecified vaccination windows at 0 months (enrollment visit) and 1 month and 6 months after enrollment. A total of 3736 women received Havrix, and 3726 women received Cervarix [6]. When the last vaccine dose was administered, at 6 months, women who were sexually active provided a self-collected specimen for HPV testing [23].

Follow-up included annual collection of cervical samples for HPV and cytological testing. Abnormal cytological findings (defined by the Bethesda system) of low-grade squamous intraepithelial lesions (LSIL) or atypical squamous cells of undetermined significance (ASC-US) necessitating triage for HPV testing [25] shifted clinical visits to a 6-month schedule; if 3 consecutive negative results of cytological tests were obtained, annual visits resumed. Participants were referred to undergo colposcopy for a cytological diagnosis of high-grade squamous intraepithelial lesions (HSIL), atypical squamous cells (cannot exclude HSIL), or glandular abnormalities and for repeat cytological testing of LSIL or HPV-positive ASC-US [6, 23].

Exfoliated cervical cells were obtained using a Cervex-Brush (Rovers Medical Devices, Oss, the Netherlands) directly applied to the cervix and eluted into PreservCyt medium (Cytyc, Marlborough, Massachusetts) for cytological and HPV testing as previously described [6].

HPV Variant Study Design

Infecting HPV types were selected based upon phylogenetic relatedness to vaccine types and/or previous evidence for partial protection or observed enhanced susceptibility to infection after immunization [6, 13, 26–31]. A total of 7 high-risk HPV types were evaluated: HPV-16 and HPV-18; HPV-31, -33, and -35 (3 types related to HPV-16 in the alpha-9 species); HPV-45 (a type in the alpha-7 species related to HPV-18); and HPV-51 (a type from the alpha-5 species) [3].

Incident infection(s) were eligible for selection, defined as an HPV infection that was detected after vaccination and not present at either enrollment (time of first vaccine dose) or the 6-month visit. Infection duration was estimated as the interval between the initial HPV-positive test result and the time of the last positive HPV test. Long-term/persistent infections were defined as incident infection(s) that lasted for >2 years worth of visits (ie, >660 days) and/or resulted in a CIN 2/3 diagnosis. Short-term/transient infections were defined as those lasting <2 years and not associated with CIN 2/3. For efficiency, all incident persistent infections/CIN 2/3 were selected, whereas a random sampling of the more abundant incident transient infections matched by HPV type were selected in a ratio of 2 short-term infections for each long-term infection. Selection for testing was masked to vaccine arm and number of doses administered.

Laboratory Characterization of HPV Type–Specific Variant Lineages

The laboratory was blinded to all data except HPV type. Briefly, DNA was isolated from exfoliated cells, and HPV genomes were assigned to variant lineages by DNA sequence analyses as previously described [17, 32]. Polymerase chain reaction (PCR) analyses targeted the upstream regulatory region (URR) and/or E6 open reading frame (ORF), because these regions allow classification of HPV variant genome lineages that are also correlated with the major polymorphisms in the L1 and L2 ORFs [15, 33–35]. Samples for which the URR assay did not yield amplification underwent repeat analysis using the E6 assay. PCR primers and conditions are provided in Supplementary Table 1 [17, 33, 36].

To classify HPV-31 lineages for the extended study, an additional PCR assay (L1_FG) amplifying a smaller fragment was developed, based on lineage-specific sequence differences in a hypervariable region of the L1 encoding the FG surface loop [33]. Classification of HPV-31 variants by the L1_FG assay was validated using samples previously characterized by the URR/E6 assays (κ = 0.95; Supplementary Table 2). Samples characterized by at least 1 HPV-31 variant assay were included in the HPV-31 extended analysis; samples with discordant variant results were excluded (n = 9). Thus, there was a difference in the number of HPV-31 infections classified as persistent infections/CIN 2/3 in the main and extended studies.

Statistical Analysis

The lineage-specific VE was calculated for each variant group. All incident persistent infections/CIN 2/3 for the described types and a 2:1 match by type for incident transient infections were included in the analysis. Since the total numbers of women at risk for the end points in the 2 vaccine arms (Cervarix and Havrix) were practically the same for each analysis, we used event counts instead of attack rates. The estimated HPV VE against viral variant lineages for each HPV type was calculated as the ratio of the difference in numbers of variant lineage end points observed between the Havrix arm and the Cervarix arm, divided by the number of events in the Havrix arm.

The expected number of events in the Cervarix arm is a binomial variable, and the sample size is equal to the total number of events in the two arms. Noting that the VE is the complement of the ratio of attack rates in the two arms, we transformed the estimates of the proportions and the confidence bounds to obtain a corresponding confidence interval (CI) for the VE, using the Clopper–Pearson method [37].

Additionally, we tested whether the efficacy of Cervarix varied by variant lineages within the same viral type, separately for each end point (ie, persistent infections/CIN 2/3 and transient infections). The ratio of the attack rates was evaluated by a Poisson test for rates, using the rateratio.test package in R [38]. The null hypothesis of the tests is equal VEs for each viral lineage within a given HPV type.

HPV-31 Extended Analysis: Sample Selection and Variant Detection

To follow-up on a suggestive initial finding in the main analysis, all HPV-31 infections were tested or retested; infections detected within 180 days of enrollment were considered prevalent and used for exploratory analyses.

RESULTS

The study design is shown in Figure 1. A total of 1106 incident infections were selected for lineage characterization of high-risk HPV types 16, 18, 31, 33, 35, 45, and 51 (Supplementary Table 3). Of the 1106 incident HPV infections selected, 842 (76.1%) were successfully characterized for variant lineage
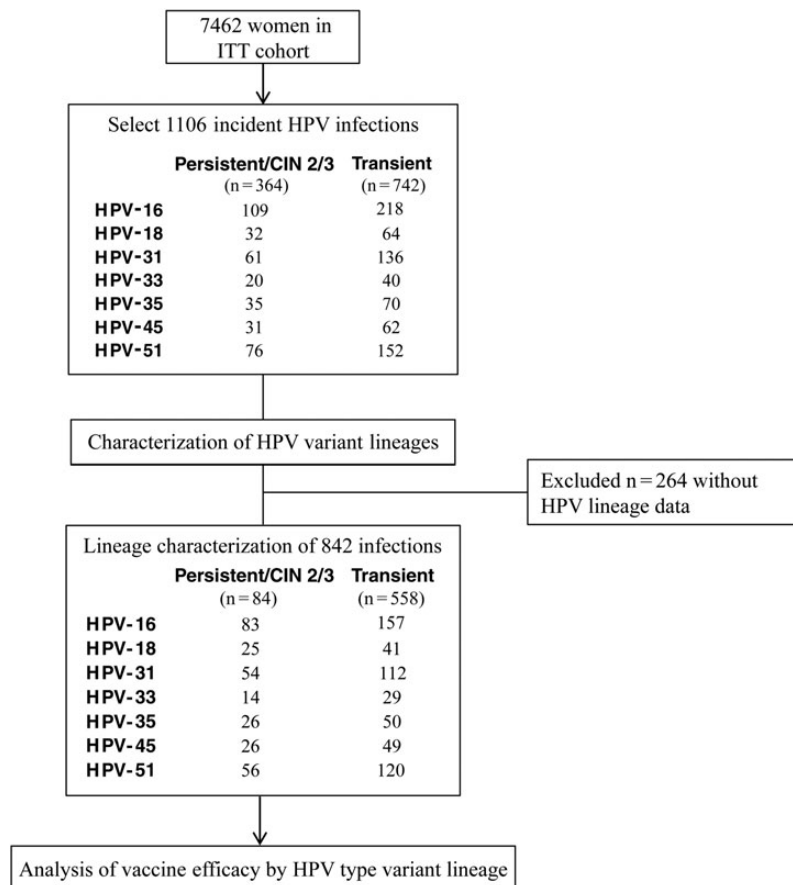

Figure 1. Study design to determine the relative efficacy of bivalent human papillomavirus (HPV) vaccine against HPV variant lineages in the Costa Rica HPV Vaccine Trial (CVT). Design of sample selection for analysis of incident HPV infections in a subset of women in the intention-to-treat (ITT) cohort of the CVT. This study was designed to assess whether the HPV vaccine relative efficacy differed for incident type-specific variant infections, using 2 different outcomes: (1) transient infection and (2) persistent infection and/or cervical intraepithelial neoplasia grade 2/3 (CIN 2/3)–associated infection (see "Materials and Methods" section).

classification; 264 infections (23.9%) were excluded either because the sample was unavailable (n = 47) or the PCR reactions failed (n = 217). Of the 842 infections characterized, 284 (33.7%) were persistent/CIN 2/3, and 558 (66.3%) were transient.

To assess whether differences in type-specific VE resulted from differences in HPV variant lineage infections, the genomes were partially sequenced to classify variant lineages, categorized by infection outcome and vaccine arm, and compared with respect to lineage-specific VE. For vaccine types HPV-16 and HPV-18, there was strong protection upon vaccination, irrespective of variant lineage or infection duration (Table 1). We next assessed vaccine variant efficacies for HPV-31, -33, -35, -45, and -51 (Table 1). Insufficient variant heterogeneity for HPV-33 and HPV-35 limited estimation of VE by lineage. Although HPV-45 infections showed cross-protection in the CVT [6], no difference was observed in comparison of lineages A (n = 35) and B (n = 40) for VE among transient infections (P = .52) or persistent infections/CIN 2/3 (P = .99; Table 1).

Incident HPV-51 infections were previously found to be increased in the Cervarix arm, compared with the Havrix arm, of the CVT [6]. Infection with this relatively common type was due to lineage A variants in 85.2% of cases (n = 150) and due to B variants in 14.8% (n = 26); no statistically significant differences for VE were observed for either persistent infections/CIN 2/3 (P = .46) or transient infections (P = .80) by lineage. Lineage A infections were more common in persistent infections/CIN 2/3 after vaccination, whereas lineage B infections were more common in transient infections after vaccination; the interaction was not statistically significant (Table 1).

HPV-31 infections were dichotomized into variant lineages A/B or C, representing the divergence of the lineages from a most recent common ancestor [33]. In women who received Cervarix and developed HPV-31 persistent infection/CIN 2/3, VE against HPV-31 variant lineage C infections was 86.4% (95% CI, 65.1%–97.1%), compared with −7.1% (95% CI, −33.9% to 0%) for HPV-31 A/B variant lineage infections (P = .02; Table 1). No significant difference was observed for VE against transient HPV-31 infection by variant lineage.

Because of the intriguing difference between persistent infections/CIN 2/3 and transient infections due to HPV-31, a post hoc study was performed to test all incident HPV-31 infections (Figure 2). An additional PCR assay was developed to characterize HPV-31 variant lineages by amplifying and sequencing a small fragment within the L1 FG loop (see "Materials and Methods" section). Seventy-four HPV-31 infections (9.2%) that were unamplified by all 3 HPV-31 variant assays were excluded.

To determine whether differences in infection duration modify the effect of vaccination on HPV-31 variants, the number of HPV-31 positive tests during follow-up was used to categorize infection periods: transient single infections, transient multiple infections, or persistent infections/CIN 2/3 (Table 2). The terms "single" and "multiple" refer to the number of HPV-31–positive tests detected within the <2-year period. The end point of ≥2 years for persistent infection was the same as that used in the main study, although the numbers are slightly different, owing to use of an additional assay.

The majority of HPV-31 infections (any lineage) were transient, and, among these, no significant difference was observed in lineage-specific VE, whether for single or multiple transient infections (Table 2). As previously observed, persistent infections/CIN 2/3 due to HPV-31 displayed significant differences by variant lineage. Incidence of persistent infections/CIN 2/3 due to HPV-31 A/B was similar by vaccine arm (17 infections in the Cervarix arm and 16 in the Havrix arm), resulting in a VE of −6.3% (95% CI, −.15% to 30.2%), whereas the VE against HPV-31C (4 infections in the Cervarix arm and 25 in the Havrix arm) was 84.0% (95% CI, 63.9%–95.5%; P = .017).

To determine whether the vaccine influenced the natural history of existing HPV-31 infections, we tested 266 infections that were prevalent at the time of vaccination. No lineage-specific effect on infection duration was observed among the prevalent HPV-31 infections (data not shown). To evaluate an HPV-naive group that should have maximum protection from Cervarix, we limited the analyses to women who received 3 vaccine doses and were negative for HPV by PCR and HPV-16/18 serologic analysis at baseline. HPV-31 results were similar as reported above (13 transient HPV-31 A/B infections in the Cervarix arm and 52 in the Havrix group, with a VE of 75%; 9 and 47 transient HPV-31 C infections, respectively, with a VE of 81%; 3 and 4 persistent infections/CIN 2/3 due to HPV-31 A/B, respectively, with a VE of 25%; and 0 and 5 persistent infections/CIN 2/3 due to HPV-31 C, respectively, with a VE of 100%).

DISCUSSION

This study evaluated whether the partial protection against or increased susceptibility to some high-risk HPV types after administration of the HPV-16/18 AS04-adjuvanted vaccine could be due to viral type genetic heterogeneity. The evolution of HPV genomes has resulted in the emergence of stable viral variant lineages with highly correlated nucleotide changes across the viral genome [15, 35]. To determine whether synthetic selective pressure created by the recent introduction of HPV vaccines limits the dispersion of specific HPV variants, we used specimens and data from the ITT cohort of the CVT. We selected HPV types previously shown to be significantly protected, partially protected, or increased after HPV vaccination in either the CVT [6, 24] or other studies [12]. We chose our end point based on the most important predictors of cancer risk following infection, viral persistence and/or CIN 2/3. We observed moderate-to-high VE against variants of HPV-31 lineage C for both transient infections (VE, 61%) and persistent infections/CIN 2/3 (VE, 84%), whereas, lineages A/B showed only partial protection for infections that were transient (VE, 58%) but not for

Table 1. Relative Vaccine Efficacy (VE) Against Incident Infection With High-Risk Human Papillomavirus (HPV) Variant Lineages

Species, Lineage, Arm	Transient Infection			Persistent Infection and/or CIN 2/3 Progression			Total Evaluated, No.
	No. (%)	Lineage-Specific VE, (95% CI)	P Value[a]	No. (%)	Lineage-Specific VE, (95% CI)	P Value[a]	
HPV-16 (alpha-9)			.97			.99	
Lineage A							
Cervarix	16 (51.6)	88.1 (81.5–93.1)		15 (48.4)	76.2 (63.8–86.0)		31
Havrix	135 (68.2)	. . .		63 (31.8)	. . .		198
Lineage D							
Cervarix	1 (50.0)	80.0 (28.4–99.5)		1 (50.0)	75.0 (19.4–99.4)		2
Havrix	5 (55.6)	. . .		4 (44.4)	. . .		9
HPV-18 (alpha-7)			.99			.93	
Lineage A							
Cervarix	2 (40.0)	92.0 (74.0–99.0)		3 (60.0)	66.7 (29.9–92.5)		5
Havrix	25 (73.5)	. . .		9 (26.5)	. . .		34
Lineage B							
Cervarix	1 (33.3)	92.3 (64.0–99.8)		2 (66.7)	81.8 (48.2–97.7)		3
Havrix	13 (54.2)	. . .		11 (45.8)	. . .		24
HPV-31 (alpha-9)			.68			.02	
Lineages A/B							
Cervarix	19 (55.9)	50.0 (33.4–66.6)		15 (44.1)	−7.1 (−33.9 to 0)		34
Havrix	38 (73.1)	. . .		14 (26.9)	. . .		52
Lineage C							
Cervarix	15 (83.3)	62.5 (45.8–77.3)		3 (16.7)	86.4 (65.1–97.1)		18
Havrix	40 (64.5)	. . .		22 (35.5)	. . .		62
HPV-33 (alpha-9)							
Lineage A							
Cervarix	10 (66.7)	44.4 (21.5–69.2)		5 (33.3)	37.5 (8.52–75.5)		15
Havrix	18 (69.2)	. . .		8 (30.8)	. . .		26
Lineage B							
Cervarix	0 (0.0)	. . .[c]		0 (0.0)	. . .[c]		0
Havrix	1 (50.0)	. . .		1 (50.0)	. . .		2
HPV-35 (alpha-9)			.34				
Lineage A							
Cervarix	18 (58.1)	25.0 (9.77–46.7)		13 (41.9)	0.0 (.0–24.7)		31
Havrix	24 (64.9)	. . .		13 (35.1)	. . .		37
Sublineage A2							
Cervarix	1 (100.0)	85.7 (42.1–99.6)		0 (0.0)	. . .[c]		1
Havrix	7 (100.0)	. . .		0 (0.0)	. . .		7
HPV-45 (alpha-7)			.52			.99	
Lineage A							
Cervarix	4 (44.4)	78.9 (54.4–93.9)		5 (55.6)	28.6 (3.67–71.0)		9
Havrix	19 (73.1)	. . .		7 (26.9)	. . .		26
Lineage B							
Cervarix	8 (61.5)	55.6 (30.8–78.5)		5 (38.5)	44.4 (13.7–78.8)		13
Havrix	18 (66.7)	. . .		9 (33.3)	. . .		27
HPV-51 (alpha-5)			.46			.80	
Lineage A							
Cervarix	51 (61.4)	0.0 (.0–6.98)		32 (38.6)	−100.0 (−79.4 to −1.0)		83
Havrix	51 (76.1)	. . .		16 (23.9)	. . .		67
Lineage B							
Cervarix	12 (75.0)	−100.0 (−54.1 to −100.0)		4 (25.0)	0.0 (0.0–60.2)		16
Havrix	6 (60.0)	. . .		4 (40.0)	. . .		10

Abbreviations: CI, confidence interval; CIN 2/3, cervical intraepithelial neoplasia grade 2/3.

[a] Data denote no. of the specified type of infection and/or disease state (% of all infections per specified lineage and vaccine arm).

[b] For comparison of the equality of vaccine efficacy for each HPV variant lineage by infection duration.

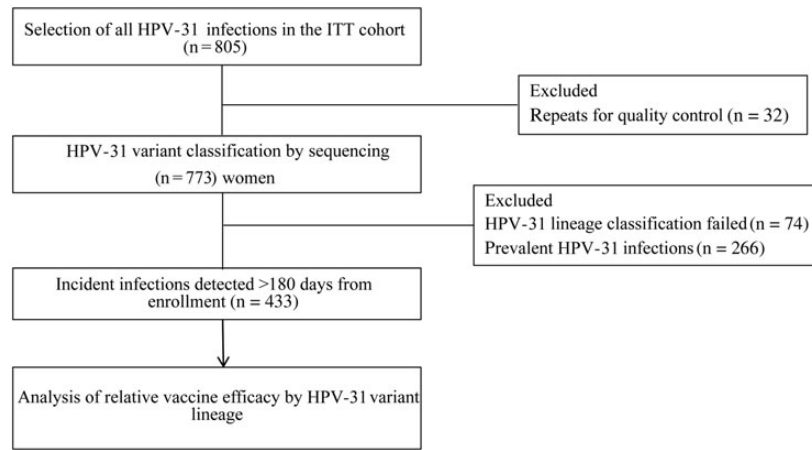[c] Not determined, because of insufficient sample size.

Figure 2. Expanded study design to assess the relative efficacy of the bivalent human papillomavirus (HPV) vaccine against HPV-31 variant infections. Samples in which HPV-31 was previously detected [6] were selected for variant lineage classification, and incident infections were evaluated for lineage-specific vaccine efficacy. A total of 433 incident HPV-31 infections, classified into variant lineages, were evaluated for relative vaccine efficacy, based on infection duration ([1] transient infection or [2] persistent infection and/or cervical intraepithelial neoplasia grade 2/3). Prevalent infections (n = 266), defined as HPV-31 detected within 180 days of study enrollment, were excluded in this analysis. Repeats for QC were an additional sample selected from a different time point in 32 women. Abbreviation: ITT, intention to treat.

those that led to persistent infection/CIN 2/3 (VE, −6%). If corroborated, this finding could be of clinical importance, since HPV-31 lineages A/B have been observed to more likely lead to development of CIN 3, compared with HPV-31 lineage C [17, 22]. We evaluated whether the difference in VE of HPV-31 A/B lineages for persistent infections/CIN 2/3 might be related to changes in duration of infection, but we did not observe a difference by vaccine arm (data not shown). Thus, it is unlikely that the vaccine acts subsequent to acquisition of an HPV-31A/B variant infection to change the natural history and/or duration of these infections [39].

In women with no prior evidence of HPV exposure, vaccination is highly efficacious against autologous HPV type acquisition and persistence [6, 13, 27, 39]. As reported in the CVT, the bivalent vaccine displayed 91% VE (95% CI, 82%–96%) against 1-year persistent HPV-16/18 and 90% (95% CI, 40%–100%) against related CIN 2/3 in the ATP cohort [24], as well as a VE of 49% (95% CI, 38%–58%) in the ITT group [6]. A more stringent end point of ≥2 years for persistence used in the current variant analysis confirmed a high efficacy against vaccine-types (HPV-16 and HPV-18) irrespective of variant lineage, maintaining high-type specific protection.

As previously reported in the CVT, VE against the HPV-16/18–related types HPV-31, -33, and -45 was 44% (95% CI, 18%–63%) in the ATP and 16% (95% CI, −5% to 32%) in the ITT group for protection against 1-year persistence [6]. Analysis by HPV genotype in the ATP revealed that only the closest phylogenetically HPV-16/18–related types, HPV-31 and HPV-45, were significantly protected [6]. Vaccine cross-protective efficacy against 6-month and 1-year persistence in the ATP arm of the CVT was significant for HPV-31, with a 1-year persistence VE of 46% (95% CI, 8%–69%). For HPV-45, only 6-month

persistence VE of 73% (95% CI, 45 to 88) was significant [6]. We observed no significant differences in VE for HPV-45 lineages A and B (Table 1).

HPV-51 1-year persistence was significantly increased in the ATP vaccine arm, with a VE of −64% (95% CI, −151% to −8%). Enigmatically, HPV-51 lineage A and HPV-51 lineage B variants were differentially increased in persistent infections/CIN 2/3 and transient infections, respectively, although VE was not statistically significantly different by lineage. In contrast, the PATRICIA trial that also used the bivalent HPV-16/18 vaccine observed weak but significant cross-protection for HPV-51 6-month persistence [13]. The reasons for the differences in VE for HPV-51 variants in this study and the HPV-51 increased incidence between studies could be related to statistical instability due to the limited number of HPV-51 lineage B infections reported here.

In a previous report, HPV-31 partial protection was associated with anti–HPV-16 antibody titers, the ability of sera to neutralize HPV-31 pseudovirions, and antibody avidity [28]. We evaluated the number of vaccine doses received by women with HPV-31 infections. There were no significant differences between the proportion of women receiving 3 versus <3 doses either by HPV-31 variant lineage (ie, A/B vs C) or by outcome, transient infection versus persistent infection/CIN 2/3 (data not shown). Thus, it is unlikely that viral titers are related to HPV-31 variant differences in partial protection for persistent infection/CIN 2/3. Theoretically, differences in protection of HPV-31 variants could result from polymorphisms within the L1 sequence that alter conformational epitopes recognized by vaccine-induced antibodies. Based on our previously reported data on the heterogeneity of HPV-31 variant complete genome sequences (supplementary figure 2A in the article by Chen et al [33]), we noted only 1 consistent L1 amino acid change

Table 2. Human Papillomavirus (HPV) Lineage–Specific Vaccine Efficacy (VE) for All Incident HPV-31 Infections in the Costa Rica HPV Vaccine Trial

Lineage, Vaccine Arm	Transient Infection (Single)[a]			Transient Infection (Multiple)[b]			All Transient Infections			Persistent Infection and/or CIN 2/3 Progression			Total Evaluated, No.[c]
	No. (%)[d]	Lineage-Specific VE, % (95% CI)	P Value[e]	No. (%)[d]	Lineage-Specific VE, % (95% CI)	P Value[e]	No. (%)[d]	Lineage-Specific VE, % (95% CI)	P Value[e]	No. (%)[d]	Lineage-Specific VE, % (95% CI)	P Value[e]	
HPV-31 A/B			.90			.76			.90			.017	
Cervarix	39 (52.0)	58.1 (47.4–68.2)		19 (25.3)	58.7 (43.2–73.0)		58 (77.3)	58.3 (49.6–66.6)		17 (22.7)	−6.3 (−.15 to 30.2)		75
Havrix	93 (60.0)	. . .		46 (29.7)	. . .		139 (89.7)	. . .		16 (10.3)	. . .		155
HPV-31 C													
Cervarix	32 (60.4)	56.8 (44.7–68.0)		17 (32.1)	66.7 (52.1–79.2)		49 (92.5)	60.8 (51.7–69.4)		4 (7.5)	84.0 (63.9–95.5)		53
Havrix	74 (49.3)	. . .		51 (34.0)	. . .		125 (83.3)	. . .		25 (16.7)	. . .		150

Abbreviations: CI, confidence interval; CIN 2/3, cervical intraepithelial neoplasia grade 2/3.

[a] Data are for infections with 1 positive HPV-31 test result.

[b] Data are for infections with >1 positive HPV-31 test result within 2 years of the previous positive test.

[c] Data are combined number of all transient infections and persistent infections and/or CIN 2/3 progression, by lineage and vaccine arm.

[d] Data denote no. of the specified type of infection and/or disease state (% of all infections per specified lineage and vaccine arm).

[e] For comparison of the equality of lineage-specific VE per variant lineage and infection duration.

difference between variants HPV-31 A/B and C lineages, and it was located in the L1 FG loop (HPV-31 amino acids L1-26; Supplementary Table 4). This conformational epitope corresponds to amino acids HPV-31 L1 262–276 and was previously shown to facilitate HPV-31 cross-reactivity and neutralization by HPV-16 [40–43] and HPV-31 [44, 45] antibodies. HPV-31 C lineage contains an alanine at L1-267, similar to that seen in HPV-16, as well as the HPV-16 L1 amino acid sequence used in Cervarix (NCBI accession no. AAC09292.1) [46]. HPV-31 A/B variants contain threonine at this position (Supplementary Table 4). Thus, it is plausible that this genetic variation could contribute to HPV-31C lineage being better protected than the A/B lineages, consistent with the enhanced activity of monoclonal antibody 31.F16 to recognize HPV-31C variant as compared to HPV-31A/B variants [45]. Nevertheless, it is not clear why this differential protection was not seen when using HPV-31 transient infections as the outcome. It is possible that persistence involves both protection and natural history differences of HPV-31 variants, as HPV-31 lineage C appears to persist longer than A/B [17, 47].

The data suggest that VE by lineage for persistent infections/ CIN 2/3 was generally lower than that observed for transient infections (eg, for HPV-45 A lineage, VE against transient infection was 79%, and VE against persistent infections/CIN 2/3 was 29%). Since the number of events was limited and because this was not an a priori hypothesis, we have not pursued this analysis in detail. It is possible that lower VE in long-term persistent infections was a result of misclassification of incident infections that were low-level prevalent infections, as discussed by Malagon et al [12].

This study has limitations. The number of infections in each outcome was limited by stratification on variant lineage. The study was well powered to detect clinically significant differences, but it does not rule out variant differences in other populations. Our study did not access rare variations in the viral variant genomes, particularly in the L1 ORF containing the neutralizing epitopes, but evaluated common changes conserved among variant lineages, based on knowledge of the evolution and conservation of HPV genetic variations.

In conclusion, we tested the hypothesis that HPV variant lineages might explain the partial protection or increased susceptibility to the HPV vaccine. Although we did not find a consistent pattern of variants having differing vaccine efficiencies, HPV-31 A/B versus C lineages showed differences in the main outcome, persistent infection/CIN 2/3. We are perplexed by these observations, as transient HPV-31 infections showed no differences. Based on the knowledge that the vaccine induces neutralizing antibodies that should affect both persistent infections/CIN 2/3 and acquisition of transient infections, we have no underlying mechanism that can explain these observations. However, data on HPV-31 variant natural history suggest that lineage C tends to be more persistent, whereas, lineages A/B are more associated with CIN 3. These observations are not

consistent with the model derived from HPV-16 variants in which persistence and oncogenicity are related [15]. Complexities in viral-host relationships beyond current understanding might be at play, or there may be random effects of population sampling yielding data that are difficult to interpret. Nevertheless, considering the enhanced oncogenicity of HPV-31 A/B lineages, this study requires confirmation or refutation in additional vaccine cohorts to determine whether HPV-31 variants show differential protection by the bivalent vaccine.

STUDY GROUP MEMBERS

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Supplementary Data

Supplementary materials are available at (http://jid.oxfordjournals.org/). Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

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