

# Persistence of an Incident Human Papillomavirus Infection and Timing of Cervical Lesions in Previously Unexposed Young Women

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## Abstract

**Background:** We analyzed data from a cohort of 553 women enrolled in the placebo arm of a randomized controlled trial of the human papillomavirus (HPV) 16/18 vaccine to study the timing of the occurrence of squamous intraepithelial lesions (SIL) or cervical intraepithelial neoplasia (CIN) following incident HPV infection and its relation to persistence of the infection.

**Methods:** At entry, women were cytologically negative, HPV 16/18 seronegative, and high-risk HPV (HR-HPV) DNA negative. Cervicovaginal samples were initially collected at 3-month and cervical samples at 6-month intervals. We estimated the mean time to SIL/CIN, relative risks of SIL/CIN following incident HPV, and odds ratios between persistent HPV and SIL/CIN.

**Results:** The mean time for SIL/CIN detection was 43.3 [95% confidence interval (95% CI), 36.4-50.1] and 46.4 (95% CI, 42.0-50.7) months from first infection with

HPV 16/18 and other HR-HPVs, respectively. Relative risks of SIL/CIN following incident HPV infection were 66.2 (95% CI, 14.9-295.1) for HPV 16/18 and 50.9 (95% CI, 11.5-225.4) for other HR-HPVs. The odds ratios of SIL/CIN for persistent HPV 16/18 infection, defined as a minimum of two and three (6 monthly) visits, were, respectively, 169.0 (95% CI, 37.2-768.6) and 169.1 (95% CI, 31.5-907.4). The majority of women with cervical infection with HPV 16/18 lasting >6 months (33 of 51, 65%) developed SIL and/or CIN.

**Conclusions:** These analyses provide the first actuarial estimate of mean time between incident HR-HPV infection in previously uninfected women and onset of cervical lesion development. Persistent HR-HPV infection, particularly HPV 16/18, is a strong predictor of cervical lesion risk and potentially a reliable end point for clinical HPV research. (Cancer Epidemiol Biomarkers Prev 2009;18(3):854-62)

## Introduction

Fifteen to 18 human papillomavirus (HPV) types are currently classified based on epidemiologic and laboratory evidence as of high oncogenic risk, with two types, HPV 16 and 18, being responsible for most of the attributable risk of cervical carcinoma (1-3). Persistent infection following acquisition of a high-risk HPV (HR-HPV) is an important early precursor event in the carcinogenic progression to cervical cancer (4-10). It is generally defined by continued detection of cervical DNA of the same HPV type (11, 12).

Infection with HR-HPV types results in a complex of cellular abnormalities of the cervical epithelium, detect-

able clinically by cytology and histopathology. HR-HPV infection is considered to generate initially productive viral infection, reflected cytologically as a low-grade lesion [atypical squamous cell undetermined significance (ASCUS)/low-grade squamous intraepithelial lesion (LSIL) with underlying grade 1 cervical intraepithelial neoplasia (CIN1); ref. 13]. The development of high-grade squamous intraepithelial neoplasia (HSIL) or CIN2-3 may follow or parallel this and progress to invasive cancer. Previous cohort studies have investigated the relationship between HPV infection and the development of cervical lesions in women (6, 8, 14-23). A systematic review of 40 studies investigating the association between persistence of HPV DNA and CIN2-3/HSIL or invasive cancer found relative risks from 1.3 [95% confidence interval (95% CI), 1.1-1.5] to 813.0 (95% CI, 168.2-3229.2), with 92% of relative risks above 3.0, despite wide variation in definition and study methodology or entry criteria (24). The magnitude of association varied by duration of persistence and testing interval. Precise standardization of HPV testing, sampling procedure, and test interval is needed for reliable clinical prediction.

We conducted a cohort analysis of participants enrolled in the placebo arm of a clinical trial of the

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HPV 16/18 vaccine in which cervical and cervicovaginal sampling, HPV DNA testing, and cytologic reporting were highly standardized (25, 26). Because trial enrollment was limited to screened young women without serologic and direct virological evidence of HR-HPV infection, an in-depth analysis of this cohort afforded a unique opportunity to study the development of persistent infection and onset of cervical lesions following incident infections as early events after first exposure to HPVs.

## Materials and Methods

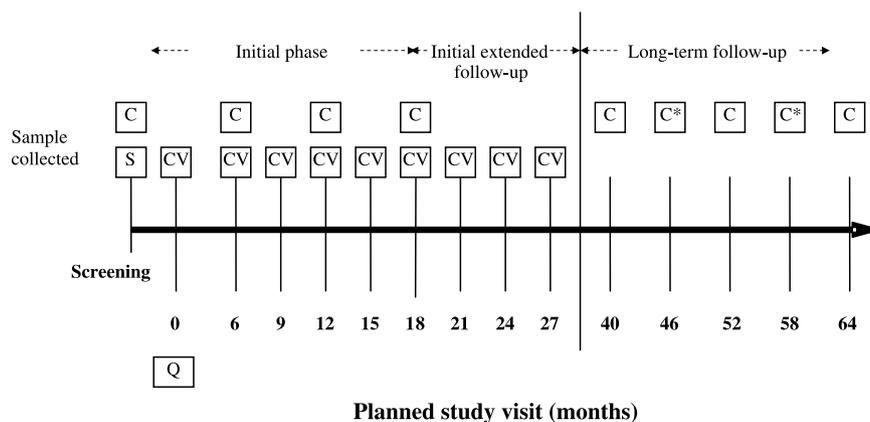
**Study Design.** A cohort of 1,113 women 15 to 25 y of age was enrolled in a double-blind, randomized, controlled trial of the HPV 16/18 L1 VLP AS04 vaccine. From this cohort, 553 women were randomized to the placebo arm of the study and received aluminum hydroxide adjuvant only. A detailed description of the design and methods of the study (25) and of its extended (blinded) follow-up phase (26) has been published previously. The aforementioned subjects enrolled in the placebo arm represented the sample used in the present analysis; the majority having completed up to 14 follow-up visits.

Briefly, healthy women were eligible to participate in the initial phase if they (a) were between 15 and 25 y of age; (b) had no more than six lifetime sexual partners; (c) had no history of an abnormal Pap test or ablative or excisional treatment of the cervix, and no ongoing treatment for external condylomata; and (d) were cytologically negative, seronegative for HPV 16 and HPV 18 antibodies by ELISA, and HPV DNA negative by a PCR assay for 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68; refs. 25, 26) at the preentry screening visit. Women eligible to participate in the extended follow-up phase participated in the initial efficacy trial and had received three doses of placebo (26).

The timeline for specimen collection is presented in Fig. 1. Health providers collected cervical samples at screening and at 6 monthly intervals during the initial and follow-up studies for HPV DNA testing. Routine screening by cytology was carried out at 6 monthly and 6 or 12 monthly intervals in the initial and extended follow-up studies, respectively. Colposcopy and biopsy were done according to protocol guidelines (25). Self-collected cervicovaginal samples for HPV testing were obtained at months 0, 6, 9, 12, 15, and 18 and at months 21, 24, and 27 in a subset. The first visit (month 0) was completed no more than 90 days after screening. The planned study visits (Fig. 1) are shown for comparability with the published vaccine trial analyses (25, 26). For statistical analysis, we calculated the actual time intervals relative to the screening visit or to the visit in which an incident HPV was documented (see below and Table 1).

Cervical specimens were collected for cytology and HPV DNA testing (cells preserved in PreservCyt, Cytyc Corp.; ref. 25). As previously described, DNA was isolated from the cervical and cervicovaginal samples and biopsy specimens. Detection and typing for 25 HPV genotypes (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74) were done using a validated algorithm developed for clinical research using the broad-spectrum PCR SPF<sub>10</sub> LiPA<sub>25</sub> system (LiPA HPV genotyping assay, SPF-10 system version 1, Innogenetics, manufactured by Labo Biomedical Products) followed by type-specific PCR for HPV types 16 and 18 (26). All HPV testing and cytologic and histologic examination was done by centralized laboratories with careful quality control and multiple observers for abnormal cytology and CIN diagnoses to take account of the recognized variation in reporting both abnormal cytology and CIN between pathologists (27).

**Statistical Analysis.** The main objective of analysis was to assess the association between incident and persistent HPV infection and incident cervical precancerous lesions, with particular attention to assessing



**Figure 1.** Timeline for specimen and data collection in the cohort study in relation to the underlying randomized controlled trial of an AS04 adjuvanted HPV 16/18 L1 VLP AS04 vaccine (25, 26). The last possible programmed visit time point for the purposes of this analysis was on month 64. The actual time distribution of visits in relation to the cohort analysis is shown in Fig. 2. *S*, serology for HPV 16/18 ELISA testing; *Q*, questionnaire for demographic and risk factor data; *C*, cervical sample collected by health provider for Pap cytology and HPV DNA testing; *C\**, cytology testing optional as a function of management algorithm; *CV*, cervicovaginal self-sample collected for HPV DNA testing only.

**Table 1. Definitions of time zero and exposure status used to estimate time to SIL/CIN in Kaplan-Meier and Cox analysis**

Incident HPV infection?	Lesion event?	Analysis layout* used in Tables 2 and 3	Definition of time zero	Definition of HPV exposure
No	No	1	Screening visit <sup>†</sup>	Negative
		2	Screening visit	Negative
Yes	No	1	Visit of incident HPV	By HPV type at time zero
		2	Screening visit	HPV positivity at the last visit
No	Yes and HPV-	1	Screening visit	Negative
		2	Screening visit	Negative
Yes	Yes and HPV- <sup>‡</sup>	1	Visit of incident HPV	Type at incident HPV event
		2	Screening visit	Negative
Yes	Yes and HPV+	1	Visit of incident HPV	Type at incident HPV event
		2	Screening visit	Type found at incident lesion

\*Corresponds to upper and lower sets of analyses shown in Tables 2 and 3.

<sup>†</sup> Preenrollment visit when subject's status was ascertained as being (a) cytologically negative, (b) seronegative for HPV 16 and HPV 18 antibodies by ELISA, and (c) HPV DNA negative by a PCR assay for 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).

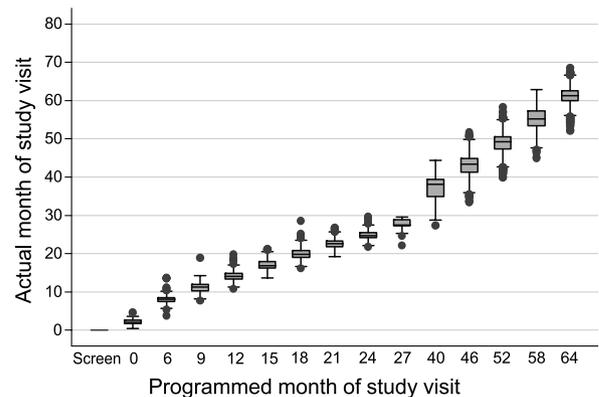
<sup>‡</sup> HPV cleared before the occurrence of lesion.

timing and the magnitude of risk for various combinations of HPV exposure. Because this data set is part of an ongoing clinical trial, all statistical analyses were done by external, independent epidemiologists (H.T., S.M.M., and E.L.F.).

We used several analytic approaches to study the natural history of cervical HPV infection and the development of cytologic and histologic end points. By definition, all virological and lesion events (with previously specified HR-HPV types) in the analyses were considered incident outcomes because all participants were HR-HPV negative by DNA testing using PCR methods and by serology, in addition to being confirmed cytologically normal at screening (Fig. 1). Outcome variables were incident SIL of low (LSIL) or high grade (HSIL; from cytologic diagnoses), or CIN (from histology). We calculated mean time to SIL/CIN and the risk of SIL/CIN according to different definitions of time zero and HPV status (Table 1). We also investigated the association between different measures of HPV persistence and risk of cervical precancerous lesions. Unless otherwise specified (see below and Table 1), we used the actual follow-up time elapsed from the screening visit (i.e., the first cytologic, serologic, or HPV DNA testing for which all enrolled women had to be negative) until the end point of interest. We used Kaplan-Meier analysis to estimate the mean time to SIL/CIN detection and the cumulative risk of SIL/CIN according to different definitions of HPV exposure (Table 1). For comparison, we also estimated the mean time to lesion based on crude (nonactuarial) analyses that did not take into account censoring by exclusively considering women who developed lesions. Using Cox proportional hazards model, we measured hazard ratios indicative of the relative risk of lesions (and respective 95% CI) in relation to HPV infection status.

Outcome variables were based on cytology alone (SIL) or cytology supplemented by biopsy histology to replace the cytologic result when available (SIL/CIN). Because of the rarity of high-grade lesions (6 HSIL assessed by cytology, or 18 HSIL/CIN2+ based on cytology/histology, including the 6 HSIL all confirmed as CIN2+) during the study period, we used any-grade SIL, for cytology-based diagnoses, or any-grade CIN in histologic assessments. Lesion incidence rates were calculated over

the accrued women-months of follow-up beginning with the screening visit or first incident HPV episode (Table 1). ASCUS events were not included as lesion outcomes. However, for the outcome based on cytology supplemented by histology, a biopsy diagnosis of CIN resulting from follow-up of an ASCUS smear was included.



**Figure 2.** Box-and-whiskers representation of the distribution of follow-up time for return visits. The boxes extend from the 25th percentile to the 75th percentile (i.e., the interquartile range); lines inside boxes represent median values. Lines emerging from boxes (i.e., the whiskers) extend to the upper and lower adjacent values. The lower adjacent value provides an estimate of the lower limit of the array and represents the first quartile value less 1.5 times the difference between the first and third quartiles. The upper adjacent value provides an estimate of the upper limit of the array and represents the third quartile value plus 1.5 times the difference between the first and third quartiles. Values outside these limits are outliers represented by dots. The number of women at each study visit was: screen, 553; 0 mo, 553; 6 mo, 518; 9 mo, 482; 12 mo, 491; 15 mo, 468; 18 mo, 478; 21 mo, 386; 24 mo, 246; 27 mo, 82; 40 mo, 382; 46 mo, 353; 52 mo, 369; 58 mo, 335; and 64 mo, 347. The study visits at the timing as designated by the study protocol at month 40 and beyond are mere approximations for comparability with the initial and extended follow-up trial data (25, 26).

**Table 2. Incidence of squamous cervical lesions (SIL or CIN) and mean time to first lesion in cohort analyses based on different exposure time and outcome definitions**

Time zero* defined at	HPV exposure <sup>†</sup> status defined at	Outcome definition	HPV exposure status <sup>‡</sup>	No. women	Women- months at risk	Incident lesions <sup>§</sup>	Mean time to lesion (mo) <sup>  </sup>	
							Actuarial (95% CI)	Crude (lesion only; 95% CI)
Screening or 1st HPV infection event	Screening or 1st HPV infection event	Cytology only	Negative	256	10,816.4	5	66.4 (65.4-67.3)	29.7 (5.5-53.8)
			Only LR-HPV types	33	868.5	4	54.5 (47.5-61.6)	10.1 (0.6-19.7)
			HR-HPV, non-16/18	111	2,886.0	30	44.8 (40.2-49.4)	18.6 (13.7-23.6)
			HPV 16 or 18	57	1,532.4	19	44.4 (37.4-51.4)	10.2 (6.3-14.2)
		Biopsy + cytology <sup>¶</sup>	Negative	256	10,908.7	2	67.1 (66.7-67.5)	55.6 (37.7-73.6)
			Only LR-HPV types	33	868.5	4	54.5 (47.5-61.6)	10.1 (0.6-19.7)
			HR-HPV, non-16/18	113	3,095.7	28	46.4 (42.0-50.7)	20.3 (15.3-25.3)
			HPV 16 or 18	59	1,620.5	21	43.3 (36.4-50.1)	12.8 (8.4-17.2)
Screening	Last visit or 1st abnormality or lesion event	Cytology only	Negative	338	15,511.1	9	67.4 (66.6-68.2)	34.9 (19.2-50.6)
			Only LR-HPV types	25	1,037.6	8	52.5 (43.7-61.2)	26.8 (11.5-42.1)
			HR-HPV, non-16/18	100	3,957.0	45	48.6 (44.1-53.0)	31.3 (26.0-36.5)
			HPV 16 or 18	59	2,120.8	32	43.4 (37.6-49.1)	29.1 (23.3-34.9)
		Biopsy + cytology <sup>¶</sup>	Only LR-HPV types	343	15,753.8	10	67.3 (66.5-68.1)	41.2 (27.6-54.8)
			Negative	25	1,118.5	7	56.3 (49.2-63.4)	35.3 (20.4-50.2)
			HR-HPV, non-16/18	95	3,895.6	40	50.6 (46.3-54.9)	33.8 (28.2-39.3)
			HPV 16 or 18	59	2,232.5	29	46.4 (41.0-51.8)	33.1 (27.4-38.8)

\*For the calculation of time to lesion event. The exit visit was the one with an incident lesion (date of incident SIL/CIN), or the last visit, for women who did not develop any lesions during follow-up. See Table 1 for details.

<sup>†</sup> By design, at the screening visit, all women were HR-HPV negative. For the first set above, HPV exposure status was based on the type found in the first HPV infection event or, if negative up to exit visit, on the status at screening. For the second set, exposure status was based on the last observation for each woman or on the HPV type associated with the incident lesion during follow-up. See Table 1 for details.

<sup>‡</sup> Does not take into account changes in status during follow-up. LR-HPV events at screening were not included as exposure outcome. For the second set, HPV-negative women may have been HPV positive at a previous instance during follow-up but cleared their infection before exit (last visit without lesion event or first instance of lesion event). See text for details on categories of HPV positivity.

<sup>§</sup>The first recorded abnormality; subsequent ones not considered.

<sup>||</sup>Whether it was based on the actuarial analysis (Kaplan-Meier estimates), which accounts for censoring, or simply restricted only to those who developed lesions.

<sup>¶</sup>Hierarchical use of cytology and biopsy diagnoses: cytology results replaced by biopsy results when biopsy was done.

Analyses of incident lesion events by HPV exposure status were based on cervical samples according to the following four exclusive and hierarchical categories: (a) negative for any of the 25 HPV genotypes detected; (b) positive only for one or more low-risk HPV (LR-HPV) types (types 6, 11, 34, 40, 42, 43, 44, 53, 54, 70, and 74); (c) positive for any HR-HPV types, except 16 or 18 (types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68); and (d) positive for HPV 16 or 18, even if another type was present.

As shown in Table 1, HPV status for lesion incidence analyses was defined in two different ways according to how HPV exposure was attributed: (a) based on the type found in the first incident HPV infection event or (b) based on the last observation for women censored at the last visit or on the HPV type associated with the first lesion event during follow-up (based on the earliest cytologic indication of an abnormality that was considered SIL or CIN on biopsy). We defined two possible starting points (time zero for Kaplan-Meier and Cox analyses) for calculating time to lesions: (a) screening visit or first incident HPV infection event, depending on whether the latter had occurred, or (b) the screening visit for all women regardless of viral events (Table 1). In all Kaplan-Meier and Cox analyses, the exit visit was the one with an incident lesion (time to event ceased to count at that point) or the last documented visit for women who did not develop any lesions during follow-up. The cytology date was used to define the time of exit visit and date of lesion incidence.

In a separate analysis, we used a cross-sectional, period-prevalence approach that considered all HPV testing and cytology results during follow-up to investigate the relation between type-specific persistent HPV and SIL/CIN. Unconditional logistic regression was used to estimate the odds ratios and their 95% CIs for the association between persistent infections and lesions. Women who tested positive for SIL/CIN at least once throughout the follow-up were defined as cases and were compared with women who remained cytologically negative throughout the study. We defined HPV persistence by taking into account the typing information in visits using (a) the cervical sample only (8 possible sampling points: at ~6 monthly intervals) or (b) the combined cervical and cervicovaginal specimens (up to 14 possible sampling points; Fig. 1). In the circumstance that combined cervical and cervicovaginal data at the same visit, a woman was considered as being infected by a specific type if either sample was positive. We classified a persistent HPV infection according to the stringency of the definition in two ways: (a) positive for HPV DNA for the same type in at least two visits (implying a duration of at least ~6 mo for analyses with cervical samples only or 3 to 6 mo for analyses with cervicovaginal specimens) and (b) positive for the same type in at least three visits (implying duration of at least 12 mo for analyses of cervical samples only and 6 to 9 mo for analyses with cervicovaginal specimens). In the first classification, transient infection was defined as having

only one visit positive for HPV DNA, whereas in the second classification, transient infection was defined as having one or two visits for HPV DNA positive even if they showed the same type. This analysis was done for the two outcome definitions: cytology only and cytology with histology. In all analyses (logistic and Cox regression), we adjusted a priori for age at baseline (<20 and ≥20 y) and ethnicity (Caucasian versus others). All analyses were done using Stata 9.2 (Stata Corp.).

## Results

A total of 553 women were enrolled into the cohort study (having received placebo). The mean follow-up time was 45.3 months (SD, 22.0) and the median follow-up time was 58.4 months (25,061.5 person-months). Figure 2 shows the distribution of the actual time elapsed since screening for each study visit and the programmed time points for the visits used for analyses. The mean age of women at entry was 20.8 years (SD, 2.7; median, 20.7; range, 15.0-25.9) and the majority were Caucasian (69.5%). Black, Hispanic, Asian, and other ethnicities represented, respectively, 7.4%, 7.2%, 1.8%, and 14.1%. Just over half of the women were recruited in North America (55.2%) and 44.8% in Brazil. The actuarial cumulative incidence of HR-HPV infections (in cervical samples) was 10%, 29%, and 52% at 12, 24, and 60 months after screening, respectively. The cumulative incidence of cervical HPV 16/18 at 60 months was 24%, of which 63% persisted for at least 6 months. Among the latter, 65% developed SIL/CIN.

Table 2 shows the actuarial and crude estimates of mean time to lesions according to timing of HPV

exposure, lesion definition, and HPV status. Following a first incident infection with HR-HPV (HR-HPV excluding HPV 16/18 or HPV 16/18), the actuarial mean time from entry to SIL/CIN detection (with HPV defined at the first lesion event) varied between 43 and 50 months, whereas the equivalent crude mean time varied between 29 and 34 months. The latter estimates are shorter than the equivalent ones from the actuarial analyses because they ignore the time elapsed among those who remain lesion-free at the time of analysis (the majority of women).

Table 3 shows the lesion incidence rates and hazard ratios according to HPV exposure for the combinations described above. All associations of SIL and CIN with HPV infection were of very high magnitude and were stronger for HR-HPV, particularly HPV 16/18. A few women (incidence, 0.5/1,000 women-months) developed SIL despite testing consistently negative in the study. Figure 3 shows the equivalent Kaplan-Meier graphs for the conservative combinations with HPV exposure defined at the time of lesion event (the two graphs represent the combinations shown at the bottom half of Tables 2 and 3).

Table 4 displays the associations between categories of HPV exposure based on persistent infection status and lesion risk defined by cytology only and by the combination of cytology and histology, respectively. The strongest associations with SIL and SIL/CIN were for persistent infection with HR-HPV, particularly with HPV 16/18. The results also suggest that associations were also stronger for lesions defined on combined biopsy and cytology results. However, even women with transient HPV infection of less than 6 or 12 months were at high risk of developing a cytohistologic lesion. Using a definition of persistence based on three HPV-positive visits of over an

**Table 3. Hazard ratios for the association between HPV exposure and squamous cervical lesions (SIL or CIN) in cohort analyses based on different exposure time and outcome definitions**

Time zero* defined at	HPV exposure <sup>†</sup> status defined at	Outcome definition	HPV exposure status <sup>‡</sup>	Incidence rate per 1,000 women-months (95% CI)	Hazard ratio <sup>§</sup> (95% CI)
Screening or 1st HPV infection event	Screening or 1st HPV infection event	Cytology only	Negative	0.5 (0.2-1.1)	1.0 (reference)
			Only LR-HPV types	4.6 (1.7-12.3)	10.1 (2.6-38.5)
			HR-HPV, non-16/18 HPV 16 or 18	10.4 (7.3-14.9)	21.0 (7.8-56.8)
		Biopsy + cytology <sup>  </sup>	Negative	12.4 (7.9-19.4)	24.6 (8.8-68.6)
			Only LR-HPV types	0.2 (0.0-0.7)	1.0 (reference)
			HR-HPV, non-16/18 HPV 16 or 18	4.6 (1.7-12.3)	28.3 (5.0-159.2)
Screening	Last visit or 1st abnormality or lesion event	Cytology only	Negative	9.0 (6.2-13.1)	50.9 (11.5-225.4)
			Only LR-HPV types	13.0 (8.4-19.9)	66.2 (14.9-295.1)
			HR-HPV, non-16/18 HPV 16 or 18	0.6 (0.3-1.1)	1.0 (reference)
		Biopsy + cytology <sup>  </sup>	Only LR-HPV types	7.7 (3.9-15.4)	14.4 (5.5-37.4)
			HR-HPV, non-16/18 HPV 16 or 18	11.4 (8.5-15.2)	19.8 (9.6-40.7)
			Negative	15.1 (10.7-21.3)	26.4 (12.4-55.8)
			Only LR-HPV types	0.6 (0.3-1.2)	1.0 (reference)
			HR-HPV, non-16/18 HPV 16 or 18	6.3 (3.0-13.1)	10.7 (4.1-28.3)
			HR-HPV, non-16/18 HPV 16 or 18	10.3 (7.5-14.0)	15.6 (7.8-31.6)
			HPV 16 or 18	13.0 (9.0-18.7)	20.8 (10.0-43.2)

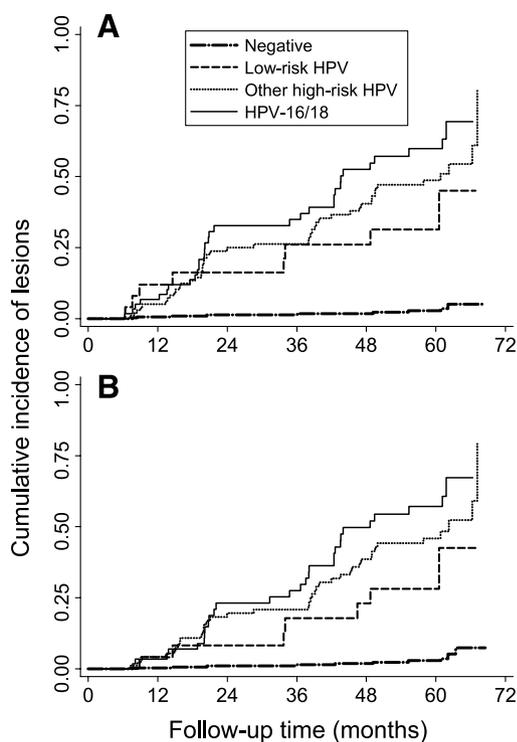
\*For the calculation of time to lesion event. The exit visit was the one with an incident lesion, or the last visit, for women who did not develop any lesions during follow-up. See Table 1 for details.

<sup>†</sup> By design, at screening, all women were HR-HPV negative. For the first set above, HPV exposure status was based on the type found in the first HPV infection event or, if negative up to exit visit, on the status at screening. For the second set, exposure status was based on the last observation for each woman or on the HPV type associated with the incident lesion during follow-up. See Table 1 for details.

<sup>‡</sup> Does not take into account changes in status during follow-up. LR-HPV events at screening were not included as exposure outcome. For the second set, HPV-negative women may have been HPV positive at a previous instance during follow-up but cleared their infection before exit (last visit without lesion event or first instance of lesion event). See text for details on categories of HPV positivity.

<sup>§</sup>Adjusted for age, race, and study region.

<sup>||</sup>Hierarchical use of cytology and biopsy diagnoses: cytology results replaced by biopsy results when biopsy was done.



**Figure 3.** Cumulative risk of developing (A) cervical any-grade LSILs (based on cytology only) and (B) cervical any-grade LSILs or CIN (based on cytology or biopsy if available) according to time zero defined at screening and HPV status defined at last visit or first lesion event. The last two combinations in Table 2 show the numbers of subjects included in the graphs.

approximate 12-month period did not substantially change the magnitude of the associations between HR-HPV and development of any SIL or CIN. The majority of women with cervical infection with HPV 16/18 persisting for >6 months (33 of 51, 65%) developed SIL and/or CIN.

## Discussion

These cohort analyses evaluated the timing and risk of development of a cytologic LSIL or CIN of any grade in relation to the occurrence and persistence of incident cervical and cervicovaginal HPV infection. The majority of infections detected were of HR-HPV, including HPV 16/18, and most lesions were low grade. All analyses show the strong association between persistence over at least two visits at 3 or 6 monthly intervals of an incident HR-HPV infection, especially HPV 16/18, and the subsequent detection of LSIL, CIN1 or worse, in young women with no evidence of previous or current cervical HPV infection.

For women who did not experience HPV infection, the risk of developing a lesion was very small. Using sensitive type-specific HPV detection, a transient HR-HPV infection of as little as 3 months or less was associated with risk of abnormality, but persistent

cervical HR-HPV infection, specifically with HPV 16/18, was a much stronger predictor of cervical lesions. Women with transient or persistent LR-HPV also had an increased risk of developing cytohistologic lesions.

The importance of persistent HR-HPV infection, including HPV 16/18, was shown regardless of exposure classification, definition of persistence, and whether cytology or histology was used. For women with such infection, the risk increased with increasing duration of persistence and numbers of 3- or 6-month visits, but it was clear that a two-visit definition of persistence was sufficient to distinguish lesion risk between very transient episodes and that resulting from infections of longer duration. Most women with persistence over 6 months developed lesions but a substantial minority of women acquired incident infections that persisted 12 months or more while remaining lesion-free during follow-up.

There has been a debate as to whether persistence precedes or is a correlate of lesion development. Findings from previous studies revealed that cytologic changes may happen within 3 months of infection and that most (around 90%) regress within 2 to 3 years (28). It has been shown that incident infections may progress to HSIL cytology or CIN2 and CIN3 within 6 to 12 months (29). The results of the present cohort study show that with appropriate actuarial estimation, the mean time from entry to initial lesion detection by cytology was 43 to 50 months, whereas the crude mean time of lesion detection was 29 to 34 months. This difference resulted from the fact that for the former estimate, we used the entire follow-up time among all women at risk, whereas the latter only considers those who actually developed lesions.

In this study, the exclusion of women with prevalent infections or lesions at entry provides assurance that the lesions resulted from first HPV exposure. Assessment of exposure is dependent on the use of a HPV DNA detection system of high molecular sensitivity for detecting multiple individual HPV types, which reduces the likelihood of false-negative HPV results and allows for the accurate identification of type-specific persistent infection. The accuracy and reliability of the HPV testing algorithm used in this study decreases the possibility for misclassification of viral exposure at entry or during follow-up (25, 26). We explored the possibility of misattribution of HPV exposure status to a lesion detected during follow-up by examining different definitions of time zero (screening or incident HPV episode) and timing for ascertaining the putatively causal HPV type (whether at the beginning of the interval or at the end). Risk correlations were of comparable magnitude in all combinations and categories of HPV exposure.

The limited sensitivity of cytology and colposcopy for the detection of CIN and the arbitrary diagnostic threshold set by the histopathologist for CIN diagnosis may all affect the timing and accuracy of the detection of lesions. As far as possible, these were standardized in this study using a central cytology and histology laboratory with multiple observers and also a colposcopy quality control program.

The strength of the observed associations and the timing of persistent HPV infection in relation to lesion

**Table 4. Odds ratios (95% CIs) for the association between HPV status and lesions in cumulative cross-sectional analysis**

Definition of cervical lesions	Specimen used to assess HPV infection	Visits included in analysis* (number to define same-type persistence)	HPV infection status	Noncases/cases	OR (95% CI) <sup>†</sup>
Cytology results only	Cervical sample only	6, 12, 18, 40, 46, 52, 58, and 64 (at least 2 visits)	Negative	199/3	1 (reference)
			Transient or persistent LR-HPV	22/6	18.8 (4.4-81.1)
			Transient other HR-HPV	44/9	12.9 (3.3-50.0)
			Persistent other HR-HPV	25/23	52.9 (14.7-190.5)
			Transient HPV 16/18	13/16	64.2 (16.3-253.0)
			Persistent HPV 16/18	15/36	148.7 (40.7-542.8)
		6, 12, 18, 40, 46, 52, 58, and 64 (at least 3 visits)	Negative	188/3	1 (reference)
			Transient or persistent LR-HPV	20/6	19.9 (4.6-86.3)
			Transient other HR-HPV	58/17	17.6 (5.0-62.5)
			Persistent other HR-HPV	9/15	87.8 (21.1-365.6)
			Transient HPV 16/18	22/38	94.0 (26.6-332.1)
			Persistent HPV 16/18	6/14	139.6 (31.3-622.1)
	Cervical and cervicovaginal self-sample <sup>‡</sup>	0, 6, 9, 12, 15, 18, 21, 24, 27, 40, 46, 52, 58, and 64 (at least 2 visits)	Negative	194/2	1 (reference)
			Transient or persistent LR-HPV	26/6	26.2 (5.0-138.8)
			Transient other HR-HPV	41/2	4.4 (0.6-32.1)
			Persistent other HR-HPV	53/27	44.6 (10.2-195.0)
			Transient HPV 16/18	30/12	31.5 (6.6-149.2)
			Persistent HPV 16/18	23/45	181.9 (41.1-806.0)
		0, 6, 9, 12, 15, 18, 21, 24, 27, 40, 46, 52, 58, and 64 (at least 3 visits)	Negative	175/2	1 (reference)
			Transient or persistent LR-HPV	25/6	24.8 (4.7-131.6)
			Transient other HR-HPV	66/8	9.6 (2.0-46.7)
			Persistent other HR-HPV	27/21	66.9 (14.6-306.5)
			Transient HPV 16/18	35/27	59.8 (13.5-265.1)
			Persistent HPV 16/18	16/30	155.3 (33.6-716.5)
Biopsy and cytology results	Cervical sample only	6, 12, 18, 40, 46, 52, 58, and 64 (at least 2 visits)	Negative	201/2	1 (reference)
			Transient or persistent LR-HPV	23/5	23.0 (4.2-127.0)
			Transient other HR-HPV	47/6	11.9 (2.3-61.3)
			Persistent other HR-HPV	26/24	76.6 (16.9-346.6)
			Transient HPV 16/18	15/15	73.1 (15.0-355.9)
			Persistent HPV 16/18	18/33	169.0 (37.2-768.6)
		6, 12, 18, 40, 46, 52, 58, and 64 (at least 3 visits)	Negative	190/2	1 (reference)
			Transient or persistent LR-HPV	21/5	24.4 (4.4-135.6)
			Transient other HR-HPV	62/14	19.9 (4.4-90.7)
			Persistent other HR-HPV	9/16	133.2 (26.0-682.2)
			Transient HPV 16/18	26/35	104.4 (23.5-463.3)
			Persistent HPV 16/18	7/13	169.1 (31.5-907.4)
	Cervical and cervicovaginal self-sample <sup>‡</sup>	0, 6, 9, 12, 15, 18, 21, 24, 27, 40, 46, 52, 58, and 64 (at least 2 visits)	Negative	195/1	1 (reference)
			Transient or persistent LR-HPV	27/5	43.6 (4.8-392.7)
			Transient other HR-HPV	42/1	4.2 (0.3-68.4)
			Persistent other HR-HPV	50/26	79.2 (10.4-600.4)
			Transient HPV 16/18	32/11	53.8 (6.7-434.8)
			Persistent HPV 16/18	28/42	276.5 (36.3-2104.3)
		0, 6, 9, 12, 15, 18, 21, 24, 27, 40, 46, 52, 58, and 64 (at least 3 visits)	Negative	176/1	1 (reference)
			Transient or persistent LR-HPV	26/5	41.2 (4.6-373.0)
			Transient other HR-HPV	69/6	13.4 (1.6-113.8)
			Persistent other HR-HPV	28/21	127.7 (16.3-1002.8)
			Transient HPV 16/18	39/26	103.4 (13.5-790.7)
			Persistent HPV 16/18	19/27	230.6 (29.4-1811.1)

NOTE: Results by unconditional logistic regression; noncases: women without lesions throughout follow-up; cases: women who developed LSIL or HSIL during follow-up (cytology-based definition, upper half of the table) or women who developed biopsy-confirmed, any-grade CIN during follow-up (biopsy and cytology results, bottom half of the table).

Abbreviation: OR, odds ratio.

\*Visits (their programmed time points) considered to define cumulative HPV and lesion status. Screening visit not considered because women were cytologically negative, seronegative for HPV 16 and HPV 18 antibodies by ELISA, and HPV DNA negative by PCR for 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). However, it is possible that women were infected at screening with a LR-HPV.

<sup>†</sup> Age and race adjusted.

<sup>‡</sup> HPV status defined as positive if either the cervical sample or the cervicovaginal self-sample was positive. See text for details on definition of HPV infection categories.

development are clinically important. Prophylactic L1 VLP vaccines have been shown to prevent infection with HPV of the target types and associated CIN lesions (30, 31). For the future development of prophylactic HPV vaccination, it is important to develop new, reproducible, validated surrogate end points that can be used to assess,

accurately, rapidly, and efficiently, vaccine efficacy and hence potential clinical effect against consequent lesions of vaccines against infection with each of the full range of oncogenic HR-HPV types.

A recent systematic review of 40 studies of the association between persistence of HPV DNA and

CIN2-3/HSIL or invasive cancer found that the variability in strength of the associations could be due to the heterogeneity across studies in definition of persistence, study methodology, and entry criteria (24). The magnitude of association varied by duration of persistence and testing interval. For persistent type-specific HPV to be acceptable as a valid and reliable end point for clinical research, precise standardization of HPV testing, sampling procedure, and test interval is needed. The present study used a standardized HPV testing system for clinical research of high analytic sensitivity and specificity for HPV detection, with cervical sampling according to a standard protocol and a central laboratory detecting cytologic and histopathologic end points in a reproducible standardized manner. The delayed median time interval between infection and lesion detection and the very high relative risks of incident HR-HPV infection persistent over 6 or 12 months or more for SIL/CIN support the use of persistence of infection as an end point that can be detected early and predicts subsequent SIL/CIN detection. This extends the evidence from the wide range of previous studies that persistent infection predicts the accepted end point of CIN2+. Further analyses to establish the relationship between incidence of individual HR-HPV types, development of persistence, and CIN2+ using this standardized methodology are planned for the placebo arms of ongoing HPV vaccine trials. The present study provides further evidence that, using a standardized approach to HPV detection, the use of HPV persistence as a clinical end point, the detection of HPV DNA on cervical samples taken 6 months apart represents a practical and valid, alternative, surrogate end point in clinical trials of vaccines.

### Disclosure of Potential Conflicts of Interest

L. Lindsay, D. Jenkins, S.L. Wieting, and A. Schuind were employed by GlaxoSmithKline while this study was conducted.

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### References

- Muñoz N, Bosch FX, de Sanjosé S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003;348:518–27.
- de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. *Virology* 2004;324:17–27.
- Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F. Carcinogenicity of human papillomaviruses. *Lancet Oncol* 2005; 6:204.
- Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999;189:12–9.
- Castle PE, Schiffman M, Herrero R, et al. A prospective study of age trends in cervical human papillomavirus acquisition and persistence in Guanacaste, Costa Rica. *J Infect Dis* 2005;191:1808–16.
- Wallin KL, Wiklund F, Angstrom T, et al. Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. *N Engl J Med* 1999;341:1633–8.
- Kjaer SK, van den Brule AJ, Paull G, et al. Type specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: population based prospective follow up study. *BMJ* 2002; 325:572.
- Schlecht NF, Kulaga S, Robitaille J, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. *JAMA* 2001;286:3106–14.
- Bosch FX, Lorincz A, Muñoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol* 2002;55:244–65.
- Munoz N. Human papillomavirus and cancer: the epidemiological evidence. *J Clin Virol* 2000;19:1–5.
- Cuschieri KS, Cubie HA, Whitley MW, et al. Persistent high risk HPV infection associated with development of cervical neoplasia in a prospective population study. *J Clin Pathol* 2005;58:946–50.
- Ho GY, Burk RD, Klein S, et al. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. *J Natl Cancer Inst* 1995;87:1365–71.
- Crum CP, McLachlin CM. Cervical intraepithelial neoplasia. *J Cell Biochem Suppl* 1995;23:71–9.
- Bory JP, Cucherousset J, Lorenzato M, et al. Recurrent human papillomavirus infection detected with the hybrid capture II assay selects women with normal cervical smears at risk for developing high grade cervical lesions: a longitudinal study of 3,091 women. *Int J Cancer* 2002;102:519–25.
- Brown DR, Shew ML, Qadadri B, et al. A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. *J Infect Dis* 2005;191:182–92.
- Castle PE, Wacholder S, Lorincz AT, et al. A prospective study of high-grade cervical neoplasia risk among human papillomavirus-infected women. *J Natl Cancer Inst* 2002;94:1406–14.
- Dalstein V, Riethmuller D, Pretet JL, et al. Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: a longitudinal French cohort study. *Int J Cancer* 2003;106: 396–403.
- Evander M, Edlund K, Gustafsson A, et al. Human papillomavirus infection is transient in young women: a population-based cohort study. *J Infect Dis* 1995;171:1026–30.
- Franco E, Villa L, Rohan T, Ferenczy A, Petzl-Erler M, Matlashewski G. Design and methods of the Ludwig-McGill longitudinal study of the natural history of human papillomavirus infection and cervical neoplasia in Brazil. Ludwig-McGill Study Group. *Rev Panam Salud Publica* 1999;6:223–33.
- Giuliano AR, Harris R, Sedjo RL, et al. Incidence, prevalence, and clearance of type-specific human papillomavirus infections: The Young Women's Health Study. *J Infect Dis* 2002;186:462–9.
- Herrero R, Schiffman MH, Bratti C, et al. Design and methods of a population-based natural history study of cervical neoplasia in a

- rural province of Costa Rica: the Guanacaste Project. *Rev Panam Salud Publica* 1997;1:362–75.
22. Moscicki AB, Hills N, Shiboski S, et al. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. *JAMA* 2001;285:2995–3002.
  23. Xi LF, Carter JJ, Galloway DA, et al. Acquisition and natural history of human papillomavirus type 16 variant infection among a cohort of female university students. *Cancer Epidemiol Biomarkers Prev* 2002;11:343–51.
  24. Koshiol JE, Lindsay L, Pimenta JM, Poole CL, Jenkins D, Smith JS. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. *Am J Epidemiol* 2008;168:123–37.
  25. Harper DM, Franco EL, Wheeler C, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet* 2004;364:1757–65.
  26. Harper DM, Franco EL, Wheeler CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet* 2006;367:1247–55.
  27. Jenkins D. Histopathology and cytopathology of cervical cancer. *Dis Markers* 2007;23:199–212.
  28. Koutsky LA, Holmes KK, Critchlow CW, et al. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. *N Engl J Med* 1992;327:1272–8.
  29. Winer RL, Kiviat NB, Hughes JP, et al. Development and duration of human papillomavirus lesions, after initial infection. *J Infect Dis* 2005;191:731–8.
  30. Ault KA; Future II Study Group. Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3, and adenocarcinoma *in situ*: a combined analysis of four randomised clinical trials. *Lancet* 2007;369:1861–8.
  31. Paavonen J, Jenkins D, Bosch FX, et al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. *Lancet* 2007;369:2161–70.

## Persistence of an Incident Human Papillomavirus Infection and Timing of Cervical Lesions in Previously Unexposed Young Women

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