Human Papillomavirus Infections and Vulvar Disease Development

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Abstract

Background: **We describe the prevalence of 14 common types [human papillomavirus (HPV)-6/11/16/18/31/33/35/39/45/51/52/56/58/59] in vulvar intraepithelial neoplasia grades 1 to 3 (VIN 1-3) and HPV genotype–specific infection in relation to the development of VIN 1-3.**

Methods: **Data were analyzed from women enrolled in the placebo arms of three randomized double-blind trials. Anogenital examinations, including collection of labial/vulvar/perineal/perianal swabs, occurred at day 1 and every 6 to 12 months through 48 months. Lesions that were possibly, probably, or definitely HPV related or of unknown etiology were biopsied. Biopsies and swabs were HPV typed. Biopsies were read for endpoint determination (VIN 1-3) by up to four pathologists.**

Results: **Incident infection with HPV-16 was the most common (6.0/100 person-years). The mean time from incident infection to the development of VIN 1-3 was 18.5 months (95% confidence interval, 13.4-23.6). HPV-6 or -11 was observed in 64.5% of VIN 1 and 29.0% of VIN 2/3, whereas HPV-16 was observed in 6.5% of VIN 1 and 64.5% of VIN 2/3.**

Conclusion: **A vaccine that includes both low- and high-risk types could prevent more than half of VIN 1-3 lesions, including the precursor lesions to HPV-related vulvar carcinoma. Understanding the incidence and duration of vulvar HPV infection and risk for progression to VIN 1-3 may inform therapeutic decisions for vulvar disease and mathematical models that assess the cost-effectiveness of vaccination.** (Cancer Epidemiol Biomarkers Prev 2009;18(6):1777–84)

Introduction

The causal role of human papillomavirus (HPV) in cervical cancer has been firmly established. HPV genotypes are categorized as having high oncogenic risk (HR), such as HPV-16 and -18, and low oncogenic risk, such as HPV-6 and -11. The advent of two prophylactic HPV vaccines, a bivalent HPV-16/18 and a quadrivalent HPV-6/11/16/18, has led to several mathematical models that have explored the acquisition of HPV infection, outcome of HPV-related disease, and natural history, as well as the cost-effectiveness of vaccination (1). For prevention of cervical disease and genital warts (for HPV-6/11/16/18 vaccination), analyses in developed world settings have consistently shown vaccination of girls and young women to have a cost-effectiveness ratio within the range typically regarded as cost-effective (2).

HPV also plays a significant role in various anogenital cancers and is an etiologic agent in nearly all warty basaloid vulvar cancers, which tend to occur in younger women (3). In contrast, squamous carcinomas occurring in older women are typically not HPV related (4). Vulvar intraepithelial neoplasia 2/3 (VIN 2/3) is considered the precursor lesion to HPV-related invasive vulvar cancers (5). In contrast to cervical disease, the development of VIN after HPV infection has not been well studied. Based on the current data on the contribution of HPV to vulvar disease, widely implemented prophylactic HPV vaccination could potentially prevent more than half of vulvar carcinomas and the respective precursor lesions (6-8). It is important therefore to understand the incidence and duration of vulvar HPV infection and the risk for progression to VIN because these data will be of value in informing therapeutic management for vulvar disease and for informing mathematical models used in assessing the cost-effectiveness of HPV vaccination. Here, we describe the incidence, duration, progression, and clearance of HPV infections in relation to the development of vulvar disease. We also present the prevalence of 14 common HPV types in VIN.

Materials and Methods

Study Design and Population. Data were analyzed from women enrolled in the placebo arms of randomized double-blind clinical trials (Merck protocols V501-011,

Received 1/22/09; revised 4/3/09; accepted 4/7/09; published online 6/8/09.

Grant Support: Merck Research Laboratories; grant support from Commonwealth Serum Laboratories and, through her institution, Merck and GlaxoSmithKline (to do clinical trials for human papillomavirus/cervical cancer vaccines; S.M. Garland); and funding through the Medical University of Vienna (E.A. Joura)

Note: Supplementary data for this article are available at Cancer Epidemiology Biomakers and Prevention Online (http://cebp.aacrjournals.org/).

The authors are responsible for the work described in this article, and all authors were involved in at least one of the following: conception, design, acquisition, analysis, statistical analysis, interpretation of data, drafting the manuscript, and/or revising the manuscript for important intellectual content.

These trials are registered at Clinicaltrials.gov (NCT00092531, NCT00092482, NCT00092534).

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doi:10.1158/1055-9965.EPI-09-0067

-012, and -015) of a quadrivalent HPV-6/11/16/18 vaccine (Gardasil, Merck and Co., Inc.). The study designs and primary results of the individual trials have been described (9-11). Institutional review boards at the participating sites approved the protocols, and written informed consent was obtained from all participants. The intensity and duration of follow-up were not related to HPV status. Subjects and investigators were blinded to treatment assignment during the course of the studies.

For each trial, women underwent comprehensive anogenital examination at each scheduled visit that included visual inspection of the perianal area, vulva, and vagina with the naked eye and a magnifying glass or a colposcope. All external anogenital and vaginal lesions that in the opinion of the investigator were possibly, probably, or definitely HPV related or whose diagnosis could not be ascertained were to be biopsied. If multiple lesions were present, the investigator was requested to obtain at least one specimen from each anatomic area affected. If, within a given anatomic area, lesions of more than one morphology were present (e.g., flat versus exophytic, hyper- or hypopigmented versus skin colored), then the investigator was instructed to biopsy a representative sample of each morphology. Biopsy samples were processed, and adjacent histologic sections of each biopsy were first read for clinical management by pathologists at a central laboratory (Diagnostic Cytology Laboratories, Indianapolis, IN) and then read for endpoint determination (VIN 1 or 2/3) by a blinded panel of up to four histopathologists. For all studies, sections of the VIN lesion were sent to a central PCR laboratory and tested for 14 HPV types (HPV-6/11/16/18/31/33/35/39/45/51/52/56/58/59; refs. 11-15). In 2004, the International Society for the Study on Vulvovaginal Disease modified the VIN nomenclature (16). Although the term VIN 1 is no longer used and VIN 2 and 3 have been replaced by "VIN usual type" and "VIN differentiated type," in this report, we maintain the original nomenclature that was used by the pathology panel during the studies.

The following genital swab specimens were obtained from all subjects across all trials: an endo/ectocervical swab (one specimen) and a combined labial/vulvar/perianal swab. In this report, ascertainment of HPV infection and progression to VIN involved HPV PCR analysis of the labial/vulvar/perineal/perianal swab. Although the three trials are similar in many ways, there are differences in the PCR analyses of the labial/vulvar/perineal/perianal swab that should be noted (Fig. 1). For all subjects in each of the three trials, the day 1 labial/vulvar/perineal/perianal swab was tested for 14 HPV types (HPV-6/11/16/18/31/33/35/39/45/51/52/56/58/59). In protocol 011, swabs collected at months 3 and 7 were analyzed for only four HPV types (HPV-6/11/16/18). In protocol 012, swabs collected at months 3 and 7 were tested for 11 HPV types (HPV-6/11/16/18/31/33/35/45/52/58/59), and those collected at months 12-18-24-30-36-42-48 were tested for nine HR HPV types (HPV-16/18/31/33/35/45/52/58/59). In protocol 015, swabs collected at month 7 were tested for only four HPV types (HPV-6/11/16/18).

Incident HPV-16/18/31/33/35/45/52/58/59 Infection. We assessed incident external genital HPV infections in the placebo arm of protocol 012 only because this was the only study wherein all swabs were tested for HPV DNA of all nine HR types. Incident external genital infection was defined by both of the following: (*a*) a positive test for one of HPV-16/18/31/33/35/45/52/58/59 on a labial/vulvar/perineal/perianal swab or external genital biopsy specimen, preceded by at least two consecutive negative swab results for the relevant type, and (*b*) no previous detection of the same HPV type in any external genital biopsy specimen obtained over the same period. For the calculation of person-time at risk, we assumed that HPV infection occurred at the midpoint in time between the positive test date and the previous negative test. A subject's person-time at risk began starting from the date of the second negative swab and was censored at the time of detection of an incident HPV infection. For individuals testing negative for a specific HPV type throughout the trial, person-time was estimated through the time point of the last labial/vulvar/perineal/perianal swab for which HPV testing was available.

Progression and Clearance of HPV-16/18/31/33/35/45/52/58/59 Infections. Incident external genital HPV-16/18/31/33/35/45/52/58/59 infections were examined until either the detection of a VIN lesion that was PCR positive for the same HPV type as the incident infection (progression), the absence of the relevant HPV DNA type in an labial/vulvar/perineal/perianal swab (clearance), or the end of the study (censoring). For progression, we used the pathology panel diagnoses from the first date at which a VIN lesion positive for the relevant HPV type was observed. When more than one grade of lesion severity was observed at the same visit, the most severe diagnosis was assigned.

Consistent with previous methods used in evaluating cervical infections and disease (17), an incident infection was considered to have cleared if a woman had two consecutive negative swab samples for a given HPV type following an incident infection or one negative sample if occurring at the last visit. Women with ongoing infection at their last observed labial/vulvar/perineal/perianal swab result were treated as censored following the date of their final swab sample. Women observed within the trial to have positive swab or biopsy specimens, followed by a single negative swab, followed by a positive swab (of the same types) or biopsy specimen, were analyzed as having persistent infection.

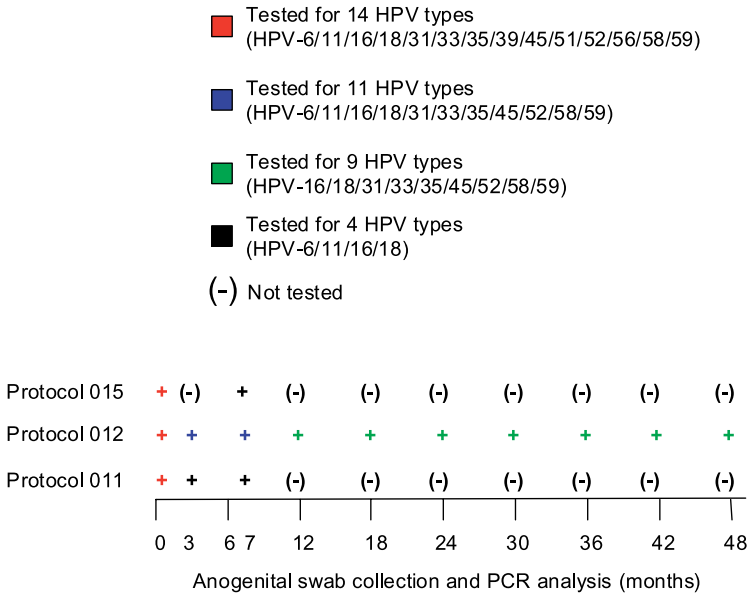
Although treatment may eradicate a VIN lesion caused by a particular HPV type, HPV infection due to that type may still be present in the normal vulvar skin following treatment, leading to persistent infection and recurrent disease (18). The persistence of external genital HPV infections was therefore evaluated from the time of VIN diagnosis for those women who had at least one labial/vulvar/perineal/perianal swab posttreatment. Women were considered to have persistent infections if their labial/vulvar/perineal/perianal swab collected posttreatment tested positive for the same HPV type that was observed in the VIN lesion.

Analysis of HPV-6/11. Per the study design, swabs were not tested for HPV-6 and -11 beyond the first three trial visits in protocol 012. Thus, it was not possible to provide direct estimates of the proportion of HPV-6 and -11 infections clearing, persisting, and progressing to VIN over time. To provide some sense for

the risk for developing VIN following incident HPV-6 or -11 infection, an alternate method from that described above for HR types was used (Supplementary Tables S1-S3).

Statistics. The cumulative proportion of HPV infections persisting without evidence of VIN, progressing, and clearing, were estimated using Kaplan-Meier methods (19). Each outcome of persistent HPV infection was mutually exclusive. Thus, once progression to VIN was observed, a woman was no longer at risk for contributing toward the cumulative rate of clearance and vice versa. Ninety-five percent confidence intervals (95% CI) for cumulative proportions persisting, progressing, and regressing were estimated through nonparametric bootstrapping of the Kaplan-Meier survivorship function with 1,000 replicates (20).

To better approximate the risk for progression for each HPV type, we did a further statistical adjustment that assumes a fractional allocation for each individual HPV type with respect to the lesion of interest when evaluating multitype infected VIN lesions (17). This was based on the relative number of instances in which each HPV type was observed as a single infection in a lesion of a given grade. For example, if one were to derive an apportionment for two VIN 1 lesions found to test positive for HPV-6 and -51, and if there were nine VIN 1 lesions with HPV-6 only, and a single VIN 1 lesion with HPV-51 only, then [2 * 9 / (9 + 1)] or 1.8 of these two multitype infected lesions would be attributed to HPV-6 and [2 * 1 / (9 + 1)] or 0.2 attributed to HPV-51.



Description of analysis	Protocols Included
Incidence of external genital HPV-6, 11, 16, 18, 31, 33, 35, 45, 52, 58 and 59 infections	012
Progression of external genital HPV-16, 18, 31, 33, 35, 45, 52, 58 and 59 infections to VIN	012
Clearance of external genital HPV-16, 18, 31, 33, 35, 45, 52, 58 and 59 infections following diagnosis and treatment of VIN	012
Progression of external genital HPV-6, 11 infections to VIN	011, 012 and 015 (see supplementary material)
Clearance of HPV-6 and 11 infections following diagnosis and treatment of VIN	Not performed due to data limitations
Prevalence of HPV-6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 infections in VIN	011, 012 and 015

Figure 1. Timing of swab collection, PCR analyses, and clinical trial populations that contributed to each of the analyses.

Prevalence of 14 HPV Types in VIN. The prevalence of 14 HPV types in VIN lesions was assessed across the three trials. We used PCR typing results from the first biopsy diagnosis of VIN for each woman to avoid selecting data for the same lesion multiple times in cases when more than one biopsy specimen was taken during the course of follow-up.

Results

Incident HPV infection, progression to VIN, and regression or clearance for HR HPV types (for progression/regression of HPV-6 and -11 infections, see supplementary material) were assessed among women enrolled in the placebo arm of protocol 012 (n = 1,795). Of these, 1,789 had HPV DNA results by PCR available for at least one labial/vulvar/perineal/perianal swab (Table 1). Approximately 65% were 20 to 23 years. Most (40.6%) were from North America. Nearly 20% reported a history of pregnancy, and 25.1% were current smokers.

Subjects in protocol 012 were followed an average of 42.2 months post day 1. The number of subjects who were eligible to contribute to the analysis of type-specific incident infection ranged from 1,546 to 1,675 (Table 2). Of the nine HR HPV types examined, incident infection with HPV-16 was the most common (6.0/100 person-years), followed by HPV-52 (3.6/100 person-years) and HPV-59 (3.5/100 person-years). Within 12 months, the proportion of HPV infections that persisted was >50% for each HPV type (Table 3), with the exception of HPV-18 (47.0%) and HPV-59 (44.0%). The proportion of HPV infections seeming to progress to VIN through 36 months (without adjudication of lesions infected with multiple HPV types) varied by type and ranged from 0.0% for HPV-33, -35, -45, and -52 to 6.0% for HPV-16. In total, 7.1% of incident infections persisted beyond 36 months without either progressing to VIN or clearing.

Across all incident infections due to HR HPV types (n = 1,196), 12 VIN lesions (1 VIN 1, 11 VIN 2/3) were diagnosed in nine women, whereas two women were diagnosed with multiple lesions (Table 4). The mean time from incident HPV infection to the development of any VIN was 18.5 months (95% CI, 13.4-23.6).

The 12 VIN lesions developing from incident infections were treated as described in Table 4. Women were considered to have persistent infections following diagnosis and treatment if their subsequent labial/vulvar/perineal/perianal swab tested positive for the same HPV type, as observed in the VIN lesion. A mean of 24.7 months of trial follow-up was available for six women. Following the detection and treatment of the incident VIN lesion, 3 (50.0%) of 6 of HPV infections cleared within 12 months. The infections that cleared were HPV-16 (2 VIN 2/3) and HPV-18 (1 VIN 2/3). The infections persisting beyond 12 months posttreatment were HPV-31 (1 VIN 1) and HPV-16 (2 VIN 2/3).

In the previous analysis, we considered only 12 VIN lesions that were diagnosed subsequent to an incident infection. To better describe the HPV types prevalent in VIN, we assessed the prevalence of 14 HPV types in VIN lesions among all women (n = 8,812) enrolled in the placebo arm of all three trials (Table 5). Ninety-three VIN lesions were diagnosed in 65 women during the follow-up period. As stated in the methods section, we determined the HPV types that were present in the first VIN biopsy specimen collected from these 65 women. For example, the HPV-31/56/59 positive VIN 2/3 that was reported in the analysis of progression to VIN (Tables 3 and 4) is not included in Table 5 because this patient was previously diagnosed with a VIN 2/3 lesion that was positive to HPV-31/58. Of the first lesions diagnosed among the 65 women, three (4.6%) lacked HPV PCR typing for 1 or more types. Of the remaining 62 VIN lesions, 31 were VIN 1 and 31 were VIN 2/3. Overall, 80.6% (25 of 31) of VIN 1 and 87.1% (27 of 31) of VIN 2/3 tested positive by PCR for $\geq$1 of the 14 HPV types. HPV-6 or -11 was observed in 64.5% of VIN 1 and 29.0% of VIN 2/3, whereas HPV-16 was observed in 6.5% of VIN 1 and 64.5% of VIN 2/3. Two (6.5%) VIN 2/3 lesions were positive for HPV-6, with no evidence of coinfection with one of the other 13 tested HPV types.

From the group of lesions testing positive for 1 or more HPV types, 14 (26.9%) of 52 had multiple HPV types detected. Of the HPV positive lesions, multiple infections were more common in VIN 2/3 (37%; 10 of 27) than VIN 1 (16%; 4 of 25); however, the differential was not statistically significant (P = 0.13). HPV-16 was the most common type observed among multiple infections (9 of 14), followed by HPV-6 (7 of 14). To better approximate the risk for progression of incident infections when multiple HPV types are present in subsequently detected VIN lesions, we did a statistical adjustment that assumes a fractional allocation for each individual HPV type with respect to the lesion of interest (Table 3), using the prevalence data from Table 5. For example, there were 12 instances wherein HPV-16 was the sole HPV type detected in a VIN 2/3 lesion, whereas HPV-11 was only observed in combination with HPV-16 (n = 2). After a statistical adjustment that assumes a fractional allocation for each individual HPV type, the 36-month risk for progression of incident HPV-16/18 infections was only slightly lower following this statistical adjudication (4.2% versus 4.5%). Table 3 shows that progression rates to VIN 2/3 seem to be highest for women with incident HPV-16 infections, followed by HPV-31, with the differential between these types of borderline statistical significance (P = 0.06).

Discussion

With the advent of two prophylactic HPV vaccines, policymakers are evaluating the population benefits and cost-effectiveness of vaccination. Essential to this is an understanding of the attribution of individual HPV types to the development of anogenital disease, including high-grade vulvar disease, as a potential precursor to vulvar cancer. Although low-grade vulvar disease is generally considered a benign event, women with a diagnosis of VIN 1 face significant therapeutic, sexual, and social consequences (21). We evaluated incident HPV infection and development of VIN among young women. The mean time from incident infection, based on the PCR analysis of swabs obtained from the labia, vulva, perineal, and perianal region, to the development of VIN was 18.5 months. Incident infection and risk for progression to VIN 2/3 was highest for HPV-16. HPV-6 was the most prevalent type observed among VIN 1 (61.3% of lesions),

Table 1. Baseline characteristics of women with labial/vulvar/perianal/perineal swab testing data

Variable	*n* (%)
Number of evaluable subjects	1,789
Age group	
16-19	629 (35.2)
20-23	1,160 (64.8)
Region	
Asia	161 (9.0)
Australia/New Zealand	151 (8.4)
Europe	330 (18.4)
South/Central America	420 (23.5)
North America	727 (40.6)
Smoking status	
Current smoker	449 (25.1)
Ex-smoker	202 (11.3)
Nonsmoker	1,138 (63.6)
Lifetime no. of sexual partners	
0	115 (6.4)
1-2	1,001 (56.0)
4-5	673 (37.6)
Past pregnancy	
Yes	337 (18.8)
No	1,452 (81.2)

consistent with their natural history. In contrast, HPV-16 was the most prevalent type observed among VIN 2/3 (64.5%). HPV 31 was the most common nonvaccine type detected in VIN 2/3. This finding is important in light of the significant partial efficacy for HPV-31 infection and cervical intraepithelial neoplasia (CIN) grade 1 or worse that has been shown for the HPV-6/11/16/18 vaccine (22). HPV types that were not observed in any of the VIN lesions in this study included HPV-35 and -45.

The present study is, to our knowledge, the first to assess the progression of incident external genital HPV infections to vulvar disease. Women were at risk for developing high-grade vulvar disease starting within the first year after incident infection. These findings are similar to previous studies on cervical disease. In a U.S. study, the median time from the first detection of an incident infection to CIN 2/3 was 14.1 months (23). Other studies have reported CIN 2/3 within 2 years of detection of HPV infection (14, 24, 25).

Several steps were taken in this trial to enhance accuracy and reproducibility. For the analysis of incident infection, swabs were collected at 6-month intervals and were analyzed for HPV DNA using a highly sensitive, type-specific PCR assay. To ensure high sensitivity for histologic endpoints, all discrete abnormal areas were to be biopsied. Thin-section microtome sections were prepared from biopsy tissue and sections adjacent to those used for histologic diagnosis were tested for HPV DNA to ensure genotyping was done on abnormal tissue. VIN lesions were obtained from women in developed and developing countries, whereas most other studies to date have examined the distribution of HPV types in VIN lesions from a single region or medical institution (4-6, 26-30).

The study is also accompanied by some limitations. Only women enrolled in protocol 012 had their swabs tested for HR HPV at all time points, thus limiting the number of women who could contribute to the analyses. The small number of incident VIN lesions observed in protocol 012 precludes the ability to detect small differences in risk between individual HPV types or to conclusively determine that the absolute risk associated with tested but nondetected types is zero. Although, we tested for 14 HPV types in all biopsies across all trials ($n = 8,812$), the prevalence of HPV DNA was estimated using PCR typing results from only the first VIN lesion observed for each woman. Thus, we may under- or overestimate the prevalence of some HPV types as compared with a study on all incident VIN lesions observed over a defined time horizon because a woman may be diagnosed with more than one incident VIN lesion over time. Finally, the time frame of our studies did not allow for the evaluation of the lifetime course of infection such that we could look at potential reactivation.

Recent studies have reported the distribution of low- and high-risk types in VIN, although there are limitations when comparing studies because of differences in HPV DNA detection methods and possible variations in HPV prevalence within individual study populations (5, 5, 27, 29, 31). A recent meta-analysis found HPV in 85.3% of VIN 2/3 and 40.4% of vulvar carcinomas (8). The most common HPV types in VIN 2/3 and vulvar carcinoma, respectively, were HPV-16 (71.9% and 32.2%), HPV-33 (8.0% and 4.5%), and HPV-18 (5.0% and 4.4%). The most common HPV types in VIN 1 were HPV-6 (22.4%),

Table 2. Incidence rates of external genital high-risk HPV infections by HPV type (protocol 012)

HPV type	No. eligible*	No. excluded (%)†	Mean exposure time, y	Cases/person-years	Incidence per 100 person-years	No. of incident VIN lesions by grade‡
16	1,546	147 (8.7)	3.0	273/4,551	6.0	8 VIN 2/3
18	1,638	55 (3.2)	3.1	121/5,130	2.4	1 VIN 2/3
31	1,607	85 (5.0)	3.1	153/4,968	3.1	1 VIN 1; 2 VIN 2/3
33	1,675	18 (1.1)	3.2	59/5,363	1.1	0
35	1,669	24 (1.4)	3.2	56/5,361	1.0	0
45	1,642	51 (3.0)	3.2	74/5,244	1.4	0
52	1,600	93 (5.5)	3.1	179/4,931	3.6	0
58	1,638	53 (3.1)	3.2	108/5,180	2.1	1 VIN 2/3
59	1,623	69 (4.1)	3.1	173/5,000	3.5	2 VIN 2/3

Abbreviation: VIN, vulvar intraepithelial neoplasia.
*Eligible women included those who had at least three trial visits with satisfactory labial/vulvar/perineal/perianal PCR test results available for each HPV type and were negative for the relevant HPV type in any external genital swab or biopsy specimens collected on or before the date of their second labial/vulvar/perineal/perianal swab. A woman is counted only once in each row. A woman may appear in more than one row.
†Excluded from the analysis because of the presence of the relevant HPV type in an external genital swab or biopsy specimen collected on or before the date of their second labial/vulvar/perineal/perianal swab.
‡The total number of VIN lesions is 12 because a single lesion may have multiple HPV types.

Table 3. Progression to VIN and clearance of incident external genital high-risk HPV infections

	Mean available observation window, mo	Proportion persisting (95% CI)	Proportion clearing (95% CI)	Nonadjudicated proportion progressing to VIN 1 (95% CI)	Nonadjudicated proportion progressing to VIN 2/3 (95% CI)	Adjudicated proportion progressing to VIN 1 (95% CI)	Adjudicated proportion progressing to VIN 2/3 (95% CI)
Pooled (n = 1,196)							
12 mo	—	56.8 (53.6-60.0)	42.5 (39.3-45.5)	0.1 (0.0-0.3)	0.7 (0.2-1.2)	0.1 (0.0-0.3)	0.6 (0.2-1.2)
24 mo	—	23.2 (20.1-26.3)	75.1 (71.9-78.2)	0.1 (0.0-0.3)	1.6 (0.8-2.5)	0.1 (0.0-0.3)	1.2 (0.5-2.0)
36 mo	—	7.1 (4.0-10.4)	90.7 (87.3-93.9)	0.1 (0.0-0.3)	2.1 (0.9-3.6)	0.1 (0.0-0.3)	1.7 (0.7-3.1)
HPV-16 (n = 273)	21.7						
12 mo	—	59.8 (53.4-65.8)	38.9 (32.6-45.3)	0.0 (—)	1.3 (0.0-2.9)	0.0 (—)	1.3 (0.0-2.9)
24 mo	—	27.1 (20.4-34.0)	69.3 (62.1-76.3)	0.0 (—)	3.6 (1.1-6.6)	0.0 (—)	3.6 (1.1-6.6)
36 mo	—	9.3 (2.9-16.3)	84.8 (77.5-91.9)	0.0 (—)	6.0 (1.5-12.0)	0.0 (—)	6.0 (1.5-12.0)
HPV-18 (n = 121)	22.5						
12 mo	—	47.0 (37.7-56.9)	52.0 (42.6-61.5)	0.0 (—)	1.0 (0.0-3.1)	0.0 (—)	0.0 (—)
24 mo	—	23.4 (14.9-32.8)	75.6 (67.1-84.5)	0.0 (—)	1.0 (0.0-3.1)	0.0 (—)	0.0 (—)
36 mo	—	9.6 (0.0-21.7)	89.4 (77.9-100)	0.0 (—)	1.0 (0.0-3.1)	0.0 (—)	0.0 (—)
HPV-31 (n = 153)	22.4						
12 mo	—	59.3 (50.5-67.2)	38.4 (30.4-46.9)	0.7 (0.0-2.1)	1.6 (0.0-4.2)	0.7 (0.0-2.1)	1.6 (0.0-4.2)
24 mo	—	27.4 (18.5-36.9)	70.3 (60.6-79.3)	0.7 (0.0-2.1)	1.6 (0.0-4.2)	0.7 (0.0-2.1)	1.6 (0.0-4.2)
36 mo	—	0.0 (0.0-12.7)	97.7 (84.8-100)	0.7 (0.0-2.1)	1.6 (0.0-4.2)	0.7 (0.0-2.1)	1.6 (0.0-4.2)
HPV-33 (n = 59)	23.4						
12 mo	—	70.3 (57.7-83.0)	29.7 (17.0-42.3)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
24 mo	—	16.9 (4.3-29.5)	83.1 (70.5-95.7)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
36 mo	—	6.3 (0.0-16.9)	93.7 (83.1-100)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
HPV-35 (n = 56)	19.5						
12 mo	—	56.0 (41.4-70.6)	44.0 (29.4-58.6)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
24 mo	—	17.2 (4.9-29.5)	82.8 (70.5-95.1)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
36 mo	—	12.9 (1.1-24.7)	87.1 (75.3-98.9)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
HPV-45 (n = 74)	20.8						
12 mo	—	55.6 (42.9-68.3)	44.4 (31.7-57.1)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
24 mo	—	27.8 (15.1-40.6)	72.2 (59.4-84.9)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
36 mo	—	5.8 (0.0-16.2)	94.2 (83.8-100)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
HPV-52 (n = 179)	20.3						
12 mo	—	63.5 (55.5-71.6)	36.5 (28.4-44.5)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
24 mo	—	27.5 (18.7-36.3)	72.5 (63.7-81.3)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
36 mo*	—	7.7 (0.0-16.2)	92.3 (83.8-100)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
HPV-58 (n = 108)	20.4						
12 mo	—	60.8 (50.3-70.3)	39.2 (29.4-49.3)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
24 mo	—	24.7 (11.6-36.3)	73.6 (61.4-86.5)	0.0 (—)	1.7 (0.0-5.6)	0.0 (—)	0.0 (—)
36 mo	—	13.7 (0.0-28.7)	84.6 (68.8-100)	0.0 (—)	1.7 (0.0-5.6)	0.0 (—)	0.0 (—)
HPV-59 (n = 173)	22.3						
12 mo	—	44.0 (35.3-52.2)	56.0 (47.6-67.4)	0.0 (—)	0.0 (—)	0.0 (—)	0.0 (—)
24 mo	—	11.7 (5.7-17.6)	86.3 (79.9-92.7)	0.0 (—)	2.0 (0.0-5.1)	0.0 (—)	0.0 (—)
36 mo*	—	3.7 (0.0-8.6)	94.3 (88.9-99.2)	0.0 (—)	2.0 (0.0-5.1)	0.0 (—)	0.0 (—)

NOTE: n is the number of incident infections. A woman is counted only once for each HPV type and may have an incident infection with more than one HPV type.
*Follow-up data for persisting infections only available through 35 and 34 months post incident HPV infection for HPV-52 and -59 infections, respectively.

HPV-16 (9.8%), and HPV-11 (9.0%). We found HPV-6 to be the most prevalent HPV type in VIN 1 (61.3% of all lesions) and HPV-16 to be the most prevalent type in VIN 2/3 (64.5%).

Other studies have reported multiple HPV types in VIN 1 and 2/3 (5, 6, 29, 30, 32). Although uncommon, multiple HPV types have been reported in ~2.8% of vulvar carcinomas (8). We did not have the opportunity to examine the HPV prevalence in invasive tumors; however, we found multiple HPV types in 32% of VIN 2/3 lesions. In most instances wherein more than one HPV type is present in a lesion, only a single type is transcriptionally active and pathogenic (5). When according a risk for progression to VIN without accounting for the presence of multiple HPV types within a lesion, it is implicitly assumed that each HPV types is individually sufficient for lesion development. This may overestimate the absolute risk for progression to VIN across HPV types. We, therefore, did a statistical adjustment that assumes a fractional allocation for each individual HPV type when evaluating multitype infected lesions. Upon elimination of double and triple counting of certain HPV types, the pooled 36-month risk for progression to VIN 2/3 for HPV-16/18/31/33/35/45/52/58/59 infections declined from 2.1% to 1.7%. The decline in the estimated 36-month risk for progression to VIN 2/3 was greatest on a relative basis for HR HPV types not directly targeted by current HPV vaccines (1.0% versus 0.3% for HPV-31/33/35/45/52/58/59). For HPV-16/18, this risk marginally declined from 4.5% to 4.2%.

With the availability of a bivalent and quadrivalent HPV vaccine, there is a need to model the natural history of HPV infection across all anogenital disease. Unlike cervical disease, accurate data about the prevalence of low- and high-grade lesions of the vulva is lacking (5, 8). In summary, we report type-specific incidence, progression, and clearance of vulvar HPV infections, as well as the distribution of HPV types in VIN 1 to 3 among women aged 16 to 23 years at study baseline, thus providing insight into the pathogenesis of vulvar neoplasia. This information should assist in analyses

Table 4. High-risk HPV type distribution across VIN lesions that arose from incident infections: per subject basis plus inclusion of the VIN treatment modality (protocol 012)

Subject no.	Lesion grade	HPV type	Treatment
1	VIN 1	31, 51*	Excision
	VIN 2/3	16, 18, 56*	Excision
2	VIN 2/3	31, 58	Procedures included topical treatment† and laser vaporization/ablation; all were done at least 4 mo postdiagnosis
	VIN 2/3	31, 56,* 59	
	VIN 2/3	16	
3	VIN 2/3	16	Withdrew from trial
4	VIN 2/3	16	Topical treatment†
5	VIN 2/3	16	Excision
6	VIN 2/3	16	Excision
7	VIN 2/3	31	Treatment was not reported within 5 mo postdiagnosis
8	VIN 2/3	16, 59	Excision
9	VIN 2/3	11,* 16	Excision

*We were unable to evaluate the time from development of incident HPV infections to progression or clearance for HPV-11, -51, and -56 because of a lack of routine testing for these HPV types on genital swabs collected throughout the study. Subject 1 was negative for HPV-51 and -56 at day 1. Subject 2 was negative for HPV-56 at day 1. Subject 9 was negative for HPV-11 at day 1, month 3, and month 7.
†The original term recorded on case report forms was "chemical ablation," which was noted as being equivalent to topical therapy.

of the potential health and economic impact of prophylactic vaccination.

Disclosure of Potential Conflicts of Interest

S.M. Garland has received advisory board fees and grant support from Commonwealth Serum Laboratories and GlaxoSmithKline, and lecture and consultancy fees from Merck and Co. S.M. Garland reports having previously owned stock in Commonwealth Serum Laboratories. S.M. Garland has received grant support through her institution from Merck and GlaxoSmithKline to do clinical trials for HPV/cervical cancer vaccines. E.A. Joura has a research contract with Merck Sharp & Dohme and GlaxoSmithKline, and has received funding through the Medical University of Vienna, together with lecture fees from Sanofi Pasteur. H.L. Sings, R. Insinga, and R.M. Haupt are employees of Merck & Co., Inc. and hold stock/stock options. Merck Research Laboratories funded this study in its entirety.

Acknowledgments

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Table 5. Prevalence of HPV-6, -11, -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, and -59 infections in VIN lesions (protocols 011, 012, and 015)

VIN 1		VIN 2/3	
Diagnosis/HPV type(s)	*n* (%)	Diagnosis/HPV type(s)	*n* (%)
Exactly one type detected	21 (67.7)	Exactly one type detected	17 (54.8)
HPV-6	17 (54.8)	HPV-6	2 (6.5)
HPV-11	1 (3.2)	—	—
HPV-16	1 (3.2)	HPV-16	12 (38.7)
—	—	HPV-31	3 (9.7)
HPV-39	1 (3.2)	—	—
HPV-56	1 (3.2)	—	—
Exactly two types detected	3 (9.7)	Exactly two types detected	8 (25.8)
HPV-6 and -11	1 (3.2)	—	—
HPV-16 and -52	1 (3.2)	—	—
HPV-31 and -51	1 (3.2)	—	—
—	—	HPV-6 and -16	2 (6.5)
—	—	HPV-6 and -31	1 (3.2)
—	—	HPV-11 and -16	2 (6.5)
—	—	HPV-16 and -33	1 (3.2)
—	—	HPV-16 and -59	1 (3.2)
—	—	HPV-31 and -58	1 (3.2)
Exactly three types detected	1 (3.2)	Exactly three types detected	2 (6.4)
HPV-6, -39, and -59	1 (3.2)	—	—
—	—	HPV-6, -16, and -31	1 (3.2)
—	—	HPV-6, -16, and -56	1 (3.2)
Negative for 14 HPV types	6 (19.4)	Negative for 14 HPV types	4 (12.9)
Total	31 (100.0)	Total	31 (100.0)

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