Progression of HPV infection to detectable cervical lesions or clearance in adult women: Analysis of the control arm of the VIVIANE study

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Key words: adult women, VIVIANE, HPV, CIN, natural history

Abbreviations: 6MPI: 6-month persistent infection; 12MPI: 12-month persistent infection; CI: confidence interval; CIN: cervical intraepithelial neoplasia; CIN1+: cervical intraepithelial neoplasia grade 1 or greater; CIN2+: cervical intraepithelial neoplasia grade 2 or greater; CIN3+: cervical intraepithelial neoplasia grade 3 or greater; FUTURE: Females United To Unilaterally Reduce Endo/Ectocervical Disease; HPV: human papillomavirus; HPV: HPV infection of any duration; HR: hazard ratio; PATRICIA: Papilloma TRIal against Cancer In young Adults; PCR: polymerase chain reaction; TVC: total vaccinated cohort; VIVIANE: Human PapillomaVirus Vaccine Immunogenicity And Efficacy

Additional Supporting Information may be found in the online version of this article.

Conflict of interest: LB, DR, FS, BG are employed by the GSK group of companies. MCB is a consultant outsourced from 4Clinics to the GSK group of companies. GD was employed by the GSK group of companies at the time of the study and has several relevant patents and received GSK shares. GD is currently a full time employee of Takeda Pharmaceuticals, Deerfield, Illinois and receives salary and stock shares. DR, FS, LB and BG own shares and stock options in the GSK group of companies. AC received research funding from Roche Molecular Systems. XC received research funding from Roche Molecular Systems. XC received research funding through his institution (ICO) from Merck & Co, SPMSD, the GSK group of companies and Gentecel. He also received honoraria for conferences from Vianex and SPMSD. GM, as investigator at a study clinical site, received fees from the GSK group of companies through her institution to do the study protocol. She also received funding from Merck Sharp & Dohme to participate as principal investigator in efficacy trials. She received travel support to attend scientific meetings, honoraria for speaking engagements and participation in advisory board meetings and consulting fees from the GSK group of companies and Merck Sharp & Dohme.

Grant sponsor: GlaxoSmithKline Biologicals SA

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DOI: 10.1002/ijc.29971

History: Received 25 Mar 2015; Accepted 13 Nov 2015; Online 19 Dec 2015

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A persistent oncogenic human papillomavirus (HPV) infection is a prerequisite for development of most cervical intraepithelial neoplasia (CIN) and cervical cancer. Together, HPV types HPV-16, HPV-18, HPV-45, HPV-31, and HPV-33 account for ~85% of invasive cervical cancer worldwide. Several determinants have been found to promote progression of oncogenic HPV infection to a CIN, including tobacco exposure, higher number of sexual partners, contraceptive use and previous pregnancy, as well as individual immune responses and infection with other sexually transmitted pathogens such as *Chlamydia trachomatis* and herpes simplex virus.

Although new HPV infections are most common in young sexually active women, women aged over 25 years remain at risk of HPV infection. Type-specific HPV infections can be redetected after a period of negativity, reflecting either persistent infection that has temporarily fallen below detectable HPV DNA levels, redetection of a potential latent infection or acquisition of a new infection. A true incident infection is more likely in the setting of new sexual partners.
Most HPV infections clear naturally. However, the natural history of clearance of a cervical HPV infection or its progression to a CIN needs to be better understood, both in young women and those aged over 25 years in order to predict likely outcomes. The control arm of prophylactic HPV vaccine trials systematically collected data on HPV types, histological lesions and potential modifiers of disease progression, and are therefore useful vehicles for such analyses. We have previously presented analyses of the natural history of HPV infection in the Pailloma TRIal against Cancer In young Adults (PATRICIA) in women aged 15–25 years.16–18 The present study describes the natural history of HPV infection, including persistence, clearance and progression to CIN in the Human PaillomaVirus: Vaccine Immunogenic ANd Efficacy (VIVIANE) study, a phase III trial of the HPV-16/18 AS04-adjuvanted vaccine (Cervarix™, GSK) in women aged over 25 years.

**Material and Methods**

This analysis was based on data collected during a 4-year follow-up period in the placebo arm of the ongoing VIVIANE trial (NCT00294047). The first participant was enrolled in February 2011. Data from the trial remain blinded. The objectives of the analysis were to investigate the risk of progression from detection of an HPV infection to detection of a CIN lesion associated with the same HPV type, or natural clearance of infection (i.e., not detectable), and to identify modifiers of these relationships.

**Study participants and procedures**

The trial methodology has been previously reported.19 Briefly, we enrolled healthy women aged over 25 years from Asia Pacific, Europe, North America and Latin America, which included a subset of up to 15% of women with a history of HPV-associated infection/disease (defined as two or more abnormal smears in sequence; abnormal colposcopy; or biopsy/treatment of the cervix). We performed HPV DNA typing every 6 months and cytological examination (Bethesda system) every 12 months using liquid-based cervical cytology samples. Women were referred for colposcopy if they had a single abnormal cytology finding of atypical squamous cell of undetermined significance, low grade squamous intraepithelial lesion associated with an oncogenic HPV type, atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesion, atypical glandular cell, and high grade intraepithelial lesion or worse. Histological classification was performed on any biopsies taken. We used a broad spectrum polymerase chain reaction (PCR) SPF10-DEIA/LiPA25 (version 1) assay to test for HPV DNA from 14 HPV oncogenic types,20 and tested oncogenic HPV-positive samples by multiplex type-specific PCR and reverse hybridisation assay to detect HPV types HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-45, HPV-52, HPV-58, and HPV-59. Women completed a similar questionnaire as previously described, asking about sexual behaviour and lifestyle factors known to influence acquisition of HPV infection.21

Written informed consent was obtained from all participants, and the protocol and other materials were approved by independent ethics committees or institutional review boards.

**Endpoint definitions and statistical analysis**

A similar analysis has been previously reported using data from the PATRICIA trial in women aged 15–25 years, the methodology of which has been reported in detail.18

**Definitions related to HPV infections**

HPV infections were classified as a transient infection (HPV DNA detected at any single point, followed by a negative sample for the same HPV type at the next evaluation, including infections detected at baseline only), 6-month persistent infection (6MPI) (same HPV type detected at two consecutive evaluations over at least a 6-month period), 12-month persistent infection (12MPI) (same HPV type detected at two consecutive evaluations over at least a 12-month period), less than 6MPI (two consecutive positive samples ≤150 days apart), and infection detected only at the last visit of the study. The time to clearance was defined as the time between the date of the first sample positive for type-specific HPV DNA and the date of the first subsequent sample negative for the type-specific HPV DNA. However, at least two typespecific negative samples taken at two consecutive intervals of ~6 months following a positive sample were required to confirm clearance. Although we recognise that apparent clearance could in reality be an inability to detect the infection, we use the term clearance for simplicity. Histologically confirmed lesions were categorised as CIN grade 1 or greater (CIN1+), CIN grade 2 or greater (CIN2+), and CIN grade 3 or greater (CIN3+). CIN1+ included CIN1, CIN2, CIN3 and adenocarcinoma in situ identified by standard methods. If more than one HPV type was found in the lesion, causality was attributed based on detection of the same HPV type in preceding samples, as previously described.22 If more than one HPV type was found in preceding samples, each infection was treated as a separate observation.

**Exposures and determinants**

The main determinants considered were HPV type (for all endpoints) and duration of detected HPV infection (clearance only). Other covariates were the cumulative tobacco exposure measured as number of pack-years (one pack-year was equivalent to 365 packs of 20 cigarettes) and as smoking history at baseline (yes or no), age at onset of the HPV infection, age at first sexual intercourse, marital/partner status, education, number of lifetime sexual partners, number of sexual partners during the 12-month period prior to the reference HPV infection, use of hormones for contraception or other indication, surgical sterilisation, use of an intrauterine device, previous pregnancy, menopausal status and history of Chlamydia trachomatis during the past 12 months.
In addition, we examined the potential effect of previous cervical HPV infection, cervical HPV co-infection, previous CIN1+ associated with an HPV type different to the reference infection (i.e., CIN1+ preceding the onset of the reference infection), concomitant CIN1+ associated with an HPV type different to the reference infection (i.e., CIN1+ following the onset of the reference infection and preceding its end) and history of HPV infection/disease or a non-intact cervix (history of cauterisation or surgical treatment involving damage to the transformation zone of the cervix). Cervical HPV co-infections and concomitant CIN1+ associated with an HPV type different to the reference HPV infection were included in the models as time-varying covariates.

Statistical analysis
The analysis was performed in the total vaccinated cohort (TVC), excluding women with high grade cytology or missing cytology data at baseline. As the trial is ongoing, some data remain blinded and are therefore not presented.

The Kaplan–Meier method and univariate and multivariable Cox proportional-hazards models were used. Hazard ratios and 95% confidence intervals (CI) were calculated. All data were censored at the last recorded visit, occurrence of an endpoint event, or at 48 months, whichever occurred first. Covariates with a p value <0.2 in the univariate model were included in the multivariable model, with the exception of region which was always included. Infections or lesions with a missing covariate value were excluded from the multivariable analysis. For lesions in which multiple HPV types were detected, each HPV type was considered as a different observation. This was also the case for the analysis of clearance.

All analyses were performed using SAS version 9.2. The analysis was performed by an external statistician to maintain the study blind.

Results
The analysis population included 2,838 women with no high grade or missing cytology data at baseline (Fig. 1a). Women who acquired an HPV infection were generally younger, had first sexual intercourse at a younger age, had more sexual partners, were more likely to smoke, were more likely to have a history of Chlamydia trachomatis infection, and were less likely to have been pregnant compared with women who did not acquire an infection (Supporting Information Table 1). Median follow-up in the study was 47.9 months.

A total of 1,073 (37.8%) women experienced 2,615 HPV infections of any duration before the last study visit; 708 (24.9%) women experienced 1,130 6MPIs and 465 (16.4%) women experienced 611 12MPIs (Fig. 1a). At baseline, 507 (17.9%) women had a prevalent HPV infection; of these, 319 (11.2%) women were subsequently identified as having a 6MPI and 214 (7.5%) as having a 12MPI (Fig. 1b). During follow-up, 888 (31.3%) women experienced an HPV infection, including 528 (18.6%) with a subsequently identified 6MPI and 311 (11.0%) with a subsequently identified 12MPI (Fig. 1c).

Risk of detecting a CIN lesion associated with a 6MPI or 12MPI
Among 708 women with 6MPI, 90 (12.7%), 49 (6.9%) and 18 (2.5%) women, respectively, had a CIN1+, CIN2+ or CIN3+ lesion associated with the same HPV type within 48 months (Fig. 1a). More CIN lesions detected following a 6MPI arose from infections first detected at baseline than from infections first detected during follow-up. Of the 319 women with a 6MPI first detected at baseline, 49 (15.3%) had CIN1+ detected, 32 (10.0%) CIN2+, and 14 (4.4%) CIN3+ (Fig. 1b). Of the 528 women in whom 6MPI was first detected during follow-up, 48 (9.1%) had CIN1+ detected, 22 (4.2%) CIN2+ and 6 (1.1%) CIN3+ (Fig. 1c).

A similar pattern was seen for the 465 women with 12MPIs, with CIN1+, CIN2+, or CIN3+ lesions associated with the same HPV type as the reference 12MPI detected in 71 (15.3%), 43 (9.2%) and 18 (3.9%) women, respectively (Fig. 1a). Again, more lesions were detected following infections first detected at baseline. Of the 214 women with a 12MPI first detected at baseline, 40 (18.7%) had CIN1+ detected, 28 (13.1%) CIN2+ and 14 (6.5%) CIN3+ (Fig. 1b). Of the 311 women in whom a 12MPI was first detected during follow-up, 34 (10.9%) had CIN1+ detected, 18 (5.8%) CIN2+ and 5 (1.6%) CIN3+ (Fig. 1c).

In the multivariable analysis of 6MPI, infection with an oncogenic HPV type was significantly associated with a higher risk of detecting a lesion (Table 1). The highest risk was observed with HPV-33, with an HR (versus a non-oncogenic HPV type) of 39.5 (95% CI: 11.7–132.9, p < 0.0001) for CIN1+ and 31.9 (8.3–122.2, p < 0.0001) for CIN2+. It was followed by HPV-16 (HR 17.9 [6.2–51.7] for CIN1+ and 21.1 [6.3–70.0] for CIN2+, p < 0.0001) (Table 1). Infection with HPV-18, HPV-31 and HPV-45 also significantly increased the risk versus non-oncogenic types of detecting CIN1+ or CIN2+ (Table 1). There was a trend for an association between the risk of detecting CIN1+ and co-infection with an oncogenic HPV type different to the reference infection or presence of a concomitant CIN1+ lesion associated with an HPV type different to the reference infection (HR: 1.5 [1.0–2.4], p = 0.067 and HR: 2.2 [0.9–5.6], p = 0.102, respectively) (Table 1). Both factors were significantly associated with the risk of CIN2+ (HR: 2.2 [1.2–4.1], p = 0.013 and 2.9 [1.2–6.8], p = 0.014, respectively) (Table 1). The analysis did not show an effect of previous cervical HPV infections or previous precancerous lesions.

Several other determinants influenced the risk of detecting lesions associated with the same HPV type as the reference 6MPI in the multivariable analysis (Supporting Information Tables 2 and 3). Peri- or post-menopausal status was associated with a lower risk of detecting a CIN1+ (HR: 0.1 [95%
Figure 1. Study flow chart: detection of HPV infections and CIN. A. Throughout study. B. Prevalent infection at baseline. C. Infection first detected during follow-up. 1Infection detected at baseline and subsequently identified as being a 6MPI or 12MPI. 6MPI: 6-month persistent infection; 12MPI: 12-month persistent infection; CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus; TVC: total vaccinated cohort.

Int. J. Cancer: 138, 2428–2438 (2016) © 2015 The Authors and GlaxoSmithKline. International Journal of Cancer published by John Wiley & Sons Ltd on behalf of UICC
CI: 0.0–0.9], p = 0.037), whilst previous pregnancy was associated with a higher risk of CIN2+ (HR 2.0 [1.1–3.7], p = 0.023). There was some indication that women aged ≥36 years at onset of the 6MPI had a lower risk of lesion detection compared with women aged 26–35 years, with HRs of 0.7 (0.4–1.0), p = 0.060 for CIN1+ and 0.6 (0.3–1.1), p = 0.090 for CIN2+. Smoking at baseline was associated nonsignificantly with an increased risk: HR: 1.3 (0.7–2.3), p = 0.369 for CIN1+ and 1.5 (1.0–3.3), p = 0.304 for CIN2+.

### Risk of detecting a CIN lesion associated with an HPV infection of any duration

Among the 1,073 women with an HPV infection of any duration, 120 (11.2%), 63 (5.9%), and 23 (2.1%), women, respectively, developed a CIN1+, CIN2+, or CIN3+ lesion.
associated with the same HPV type within 48 months (Fig. 1a). Of 507 women with an HPV infection at baseline, CIN1+, CIN2+, or CIN3+ associated with the same HPV type were detected in 63 (12.4%), 40 (7.9%), and 18 (3.6%), respectively (Fig. 1b). Of 888 women with an HPV infection detected during follow-up, CIN1+, CIN2+, or CIN3+ associated with the same HPV type were detected in 69 (7.8%), 32 (3.6%), and 7 (0.8%), respectively (Fig. 1c).

Again, infection with an oncogenic HPV type was the strongest predictor of lesion detection. HPV-33 was associated with the highest risk for CIN1+ and CIN2+, followed by HPV-18, HPV-16, HPV-31, and HPV-45 for CIN1+, and by HPV-16, HPV-18, HPV-31, and HPV-45 for CIN2+ (Table 2). Infections of at least 6 months’ duration were associated with a higher risk than infections of shorter duration for both CIN1+ and CIN2+ (Table 2). Co-infection with an oncogenic HPV type or a concomitant CIN1+ was significantly associated with an increased risk of detecting CIN1+ and CIN2+ (Table 2). The analysis did not show an effect of previous HPV infection or previous CIN1+.

Other determinants also influenced risk in the multivariable analysis. Previous pregnancy was associated with a higher risk of detecting CIN1+ (HR: 1.9 [1.3–2.9], p = 0.003) and CIN2+ (HR: 2.9 [1.6–5.5], p = 0.001), whilst perinatal status was associated with a lower risk (HR: 0.2 [0.1–0.7], p = 0.014 for CIN1+ and HR: 0.2 [0.1–1.3], p = 0.093 for CIN2+). Smoking at baseline was also associated with an increased, but nonsignificant risk of detecting CIN1+ (HR: 1.3 [0.8–2.0], p = 0.278) and CIN2+ (HR: 1.5 [0.8–2.9], p = 0.260). Women aged ≥36 years at onset of the HPV infection had a lower but nonsignificant risk compared with women aged 26–35 years of detection of CIN1+ (HR: 0.7 [0.5–1.1], p = 0.112) and CIN2+ (HR: 0.7 [0.4–1.2], p = 0.185).

**Apparent clearance of HPV infection**

A total of 851 women cleared 1,665 infections (Fig. 1a). Out of 507 women with an HPV infection at baseline and follow-up, 402 (79.3%) cleared the infection. Of 319 women with a 6MPI at baseline, 223 (69.9%) cleared the infection. Overall, there was a 77% (95% CI: 75–79) chance of clearing an HPV infection at 24 months and 89% (87–91) at 48 months.

The median duration of all HPV infections (present at baseline or detected during study follow-up) was 11.5 months. Median duration of infection was 17.4 months for HPV-31, 12.5 months for HPV-16, 12.0 months for HPV-45, 11.8 months for HPV-18, 11.7 months for HPV-33, 11.3 months for other oncogenic HPV types and 11.2 months for non-oncogenic HPV types (log rank test p = 0.006) (Fig. 2). However, the difference between HPV types was no longer significant after adjustment for other covariates.

Women who smoked at baseline were significantly less likely to clear an infection than nonsmokers (HR: 0.8 [0.7–0.9], p = 0.004). The effect of age at onset of the HPV infection was not significant in the univariate analysis and was not included in the multivariable analysis.

**Discussion**

The analysis confirmed that persistent infection with an oncogenic HPV type was the main risk factor for detecting a CIN lesion in our study population. HPV-33 and HPV-16 were associated with the highest risk, followed by HPV-18, HPV-31 and HPV-45. Compared with a 6MPI with a non-oncogenic HPV type, the risk of lesion detection was 30–40 times higher for HPV-33 and approximately 20 times higher for HPV-16. Clearance rates were high, and overall, only one tenth of HPV infections failed to clear by 4 years. The median duration of all HPV infections was ~1 year. HPV-31 had the longest duration of detectable infection, followed by HPV-16, HPV-45, and HPV-18.

These findings are consistent with other studies in a younger age group. In parallel with the present analysis, we conducted a post-hoc analysis of the cumulative incidence of lesions in women aged over 25 years in VIVIANE compared with women aged 15–25 years in the previous PATRICIA study. Overall, the risk of detecting a CIN1+ or CIN2+ following an HPV infection was similar in VIVIANE and PATRICIA (Supporting Information Fig. 1). Analyses of the PATRICIA study and the FUTURE (Females United To Unilaterally Reduce Endo/Ectocervical Disease) study of the HPV-6/11/16/18 vaccine in young women also showed that HPV-33 and HPV-16 have the strongest association with lesion detection, including CIN3+ in PATRICIA. In PATRICIA, the risk of detecting CIN1+ was approximately 4-fold higher for HPV-16 and HPV-33 versus nononcogenic HPV types, and approximately 10-fold higher for CIN2+ after 4 years. A higher risk of progression associated with HPV-16 and HPV-33 has also been shown in population-based studies. In a cross-sectional study in the Netherlands of women (30–60 years of age) participating in a cervical cancer screening programme who were infected with an oncogenic HPV type, women with CIN2+ and CIN3+ were significantly more likely to be positive for HPV-16 and HPV-33 than women with normal cytology. A case-control study in New Mexico, US showed that women of all ages positive for HPV-16 and HPV-33 had an equal risk of developing carcinoma in situ or adenocarcinoma in situ. Also in New Mexico, a surveillance programme of women of any age attending for cervical screening showed that HPV-16 and HPV-33 were the types most often detected in high-grade cytological abnormalities. In the United Kingdom, a study of women with abnormal cytology referred for colposcopy found that HPV-33 had a very high positive predictive value for CIN2+ and suggested that women with HPV-33 infections should be managed similarly to women with HPV-16 infections. A prospective, population-based study of 10,000 adult women (≥18 years) in Guanacaste, Costa Rica concluded that HPV-16 remains the most carcinogenic HPV type overall.
Although HPV-18 has a high prevalence in invasive cervical cancer, women in VIVIANE infected with HPV-18 had a lower risk of developing CIN2+ than women infected with HPV-16 or HPV-33, and a similar risk as women infected with HPV-31 and HPV-45. These findings are consistent with PATRICIA and other studies.18,26,27

| Table 2. Multivariable analysis of the risk of detecting a CIN lesion associated with the same HPV type for HPV infections of any duration |
|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **HPV type**                     | **CIN1+**        | **CIN2+**        | **CIN1+**        | **CIN2+**        | **CIN1+**        |
| Non-oncogenic type               | 2,601 infections in 1,068 women 168 lesions                  | 2,601 infections in 1,068 women 92 lesions                  |
| HPV-16                          | 26               | 23              | 11.1 (5.1–24.3) | <0.0001         | 23              |
| HPV-18                          | 13               | 8               | 11.6 (5.0–27.0) | <0.0001         | 16.7 (5.4–51.5) |
| HPV-31                          | 15               | 10              | 10.3 (4.5–23.9) | <0.0001         | 16.4 (5.1–25.9) |
| HPV-33                          | 17               | 12              | 21.8 (9.3–51.0) | <0.0001         | 31.2 (10.2–95.3) |
| HPV-45                          | 7                | 4               | 6.4 (2.2–18.4)  | 0.001           | 9.1 (2.2–37.5)  |
| Other oncogenic type             | 80               | 31              | 6.6 (3.2–13.5)  | <0.0001         | 5.6 (2.1–15.0)  |
| **Duration of infection**        |                  |                 |                 |                 |                 |
| Transient and less than 6MPI    | 57               | 28              | 1               |                 | 1               |
| 6MPI                            | 111              | 64              | 2.2 (1.6–3.1)   | <0.0001         | 2.3 (1.4–3.8)   |
| **Previous cervical HPV infection** |                  |                 |                 |                 |                 |
| No                              | 106              | 61              | 1               |                 | 1               |
| Yes (at least 1 oncogenic HPV type) | 56               | 53              | 1.2 (0.8–1.9)   | 0.343           | 1.5 (0.8–2.7)   |
| Yes (only non-oncogenic HPV types) | 6               | 2               | 0.9 (0.4–2.1)   | 0.808           | 0.4 (0.1–3.4)   |
| **Cervical HPV co-infection**    |                  |                 |                 |                 |                 |
| No                              | 69               | 37              | 1               |                 | 1               |
| Yes (at least 1 oncogenic HPV type) | 89               | 53              | 1.8 (1.2–2.6)   | 0.003           | 2.1 (1.3–3.5)   |
| Yes (only non-oncogenic HPV types) | 10               | 2               | 0.8 (0.4–1.5)   | 0.473           | 0.3 (0.1–1.4)   |
| **Previous CIN1+**              |                  |                 |                 |                 |                 |
| No                              | 160              |                 | Not included    |                 | Not included    |
| Yes (any oncogenic or non-oncogenic HPV type) | 8               |                 | NA              |                 | NA              |
| **Concomitant CIN1+**            |                  |                 |                 |                 |                 |
| No                              | 155              | 80              | 1               |                 | 1               |
| Yes (any oncogenic or non-oncogenic HPV type) | 13               | 12              | 2.8 (1.4–5.6)   | 0.005           | 3.4 (1.7–6.8)   |

1Covariates were included in the multivariable analysis if they had a global p value <0.2 in the univariate analysis (except region which was always included); covariates were: region, tobacco exposure measured as number of pack-years, age at onset of the HPV infection, age at first sexual intercourse, marital/partner status, education, number of lifetime sexual partners, number of sexual partners during the past 12 months, use of hormones for contraception or other indication, surgical sterilisation, use of an intrauterine device, previous pregnancy, menopausal status, and history of Chlamydia trachomatis during the past 12 months.

2Infections or lesions with a missing value for a covariate included in the analysis were excluded from the multivariable analysis.

3Time-varying covariate.

4CIN1+ associated with an HPV type different to the reference infection, preceding the onset of the 6MPI.

5CIN1+ associated with an HPV type different to the reference infection, concomitant to the 6MPI (following its onset and preceding its end). Values in italics show the global p value.

6MPI: 6-month persistent infection; CIN: cervical intraepithelial neoplasia; CI: confidence interval.

Int. J. Cancer: 138, 2428–2438 (2016) © 2015 The Authors and GlaxoSmithKline. International Journal of Cancer published by John Wiley & Sons Ltd on behalf of UICC
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ages, HPV-18 infection is reported to have a relatively low risk of detection in CIN, but a higher risk of subsequent progression to invasive cervical cancer, especially to adenocarcinoma.\(^{32,33}\) This may be potentially attributable to a number of factors including the anatomic distribution of HPV-18-related cancers, which may be more difficult to sample if located higher in the cervical canal.

A coinfection with an oncogenic HPV type different to the referent infection and a concomitant CIN1+ lesion increased the risk of detecting CIN1+ and CIN2+. However, the analysis did not show an effect of previous infections or previous lesions on risk of lesion detection. In PATRICIA, risk of detection was increased by concomitant infection, but not by previous infection, and was also increased by both concomitant and previous CIN1+.\(^{18}\) The role of multiple oncogenic infections in the natural history of HPV infection is controversial, with some studies showing a higher risk of acquiring a new HPV type if already infected,\(^{34,35}\) and others showing that infections with different HPV types occur independently of one another.\(^{36,37}\) Two recent studies have shown no evidence of a synergistic association of infection with multiple HPV genotypes and risk of high-grade squamous intraepithelial lesions or CIN2+.\(^{28,38}\) In addition, laser microdissection of CIN lesions has shown that each component of the lesion is associated with a single HPV type i.e., one virus causing one lesion.\(^{39}\)

Behavioural risk factors for lesion detection in an older age group may differ compared with those in a sample of younger women, as the prevalence of these behaviours changes over the lifespan. In VIVIANE, previous pregnancy increased the risk of lesion detection, and was also increased by both concomitant and previous CIN1+.\(^{18}\) The role of multiple oncogenic infections in the natural history of HPV infection is controversial, with some studies showing a higher risk of acquiring a new HPV type if already infected,\(^{34,35}\) and others showing that infections with different HPV types occur independently of one another.\(^{36,37}\) Two recent studies have shown no evidence of a synergistic association of infection with multiple HPV genotypes and risk of high-grade squamous intraepithelial lesions or CIN2+.\(^{28,38}\) In addition, laser microdissection of CIN lesions has shown that each component of the lesion is associated with a single HPV type i.e., one virus causing one lesion.\(^{39}\)

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A limitation of the analysis is that CIN1 reflects a state of infection rather than a stage in disease development. Detection of CIN1+ following HPV infection does not therefore automatically represent disease progression. Nevertheless, the CIN1+ endpoint provides valuable information on the natural history of HPV infection. In addition, apparent clearance may relate to an inability to detect the infection; clearance rates should therefore be interpreted with caution. A further limitation is that misclassification of HPV infection below the threshold for detection (a false-negative result) might have underestimated persistent infection rates. However, a very sensitive HPV PCR algorithm was used. CIN detection rates may have been underestimated because the 4-year follow-up period was not long enough to detect all lesions, especially
those associated with an HPV type with a slower rate of progression from infection to lesion. More frequent follow-up would have allowed earlier detection of events, enabling more accurate estimates of the time between detection of infection and detection of a lesion or clearance. Lastly, most lesions were detected from what could be considered prevalent infections when persistent infection was first seen at baseline. For these cases, age when the infection first occurred could not be accurately determined and indeed many may have been present from adolescence and young adulthood. This limitation may have contributed to the lack of statistically significant association of lesion detection by age group and when comparing rates of lesion detection with those in PATRICIA.

In conclusion, persistent infection with an oncogenic HPV type was the main risk factor for CIN1+ and CIN2+ detection in women aged over 25 years, with HPV-33 and HPV-16 being associated with the highest risk. Concomitant HPV infection or CIN1+ due to an HPV type different to the reference infection also increased the risk of lesion development. Compared with women aged 15–25 years in PATRICIA, the risk of CIN detection following a 6MPV or HPV1V was similar in women aged >25 years. Overall, clearance rates were high. These findings may contribute towards a better understanding of the natural history of HPV infections and CIN lesions at different ages.

**Acknowledgements**

All authors participated in the design, implementation, or analysis of the study and its interpretation, as well as development of this manuscript. All authors had full access to the data and gave final approval before submission. The corresponding author was responsible for submission of the publication. GlaxoSmithKline Biologicals SA was the funding source and was involved in all stages of the study conduct and analysis (ClinicalTrials.gov Identifier: NCT 00294047). GlaxoSmithKline Biologicals SA also took in charge all costs associated with the development and the publishing of the present manuscript. The authors would like to thank the VIVIANE study team in GSK Vaccines, Marie-Pierre David (GSK Vaccines), Alice Raillard, Sabrina Collas De Souza and Aurélie Le Plain (4Clinics on behalf of GSK Vaccines) for statistical support, Dominique Descamps (GSK Vaccines) and Karin Hardt (GSK Vaccines) for clinical support, Mary Greencare PhD (An Sgriothbadair Ltd, UK) for medical writing services provided on behalf of GSK Vaccines, and Stéphanie Delval PhD (XPE Pharma & Science) and Jenny Anderson (CROMSOURCE, London, UK), on behalf of GSK Vaccines for publication coordination and management and ensuring fulfillment of ICMJE recommendations. They thank all study participants and the VIVIANE Investigators involved in this study including R Verheijen, Jorge Salmeron, H Kittchener, J Stapleton, EH Quck, M Martens, M Cruijssink, G Girard, C Bouchard, KL Fong, A Ilancheran, V Patricio, K Julien, TM Stoney, M Ferguson, A Cruz, H Gomez Moreno, A Savitcheva, N Savani, C Chambers, P Fine, B Fox, J Hedrick, J Rosen, M Sperling, S Angsuwathana, A Tristram, B ter Harmel, L Ferguson, T Poling, N Ilini, N Chakhtoura, W Utian, C Hansen and I. Leeman. We particularly acknowledge the role that Anne Szarewski played in the VIVIANE study before her untimely death. Cervarix is a trademark of the GSK group of companies.

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Title:
Progression of HPV infection to detectable cervical lesions or clearance in adult women: Analysis of the control arm of the VIVIANE study

Date:
2016-05-15

Citation:
Skinner, S. R., Wheeler, C. M., Romanowski, B., Castellsague, X., Lazcano-Ponce, E., Rowena Del Rosario-Raymundo, M., Vallejos, C., Minkina, G., Da Silva, D. P., McNeil, S., Prilepskaya, V., Gogotadze, I., Money, D., Garland, S. M., Romanenko, V., Harper, D. M., Levin, M. J., Chatterjee, A., Geeraerts, B. ..., Baril, L. (2016). Progression of HPV infection to detectable cervical lesions or clearance in adult women: Analysis of the control arm of the VIVIANE study. INTERNATIONAL JOURNAL OF CANCER, 138 (10), pp.2428-2438. https://doi.org/10.1002/ijc.29971.

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