# HUMAN PAPILLOMAVIRUS VACCINATION IN GIRLS AND WOMEN LIVING WITH HIV

by

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## ABSTRACT

**Background:** Human papillomavirus (HPV) is the causal agent of virtually all cervical cancer and genital warts. Women living with HIV (WLWH) experience higher rates of HPV-associated infection and disease than women without HIV. HPV vaccination has proven safe and efficacious in young women without HIV, however, little was known about the vaccine in WLWH. The work represented in this thesis was designed to answer key questions around HPV infection and the impact of HPV vaccination in WLWH.

**Methods:** WLWH across Canada were invited to participate in a CIHR-funded, multi-centre study of quadrivalent HPV vaccination starting in 2009. Participants were administered three doses of vaccine at 0/2/6 months. Demographic and clinical data, serology (cLIA), liquid-based cervical cytology, and HPV DNA genotyping (Linear array assay) were collected at baseline and post-vaccine series every 6-12 months up to 8 years. Participants were referred for clinical colposcopies as per the standard at their institutions.

**Results:** Pre-vaccination rates of prevalent and persistent oncogenic HPV infection among participants were high. Extending the spacing of the three vaccine doses out to two years did not significantly impact the peak anti-HPV antibody titer achieved in this cohort. Two years post-vaccination, efficacy of the vaccine was good, demonstrating lower rates of clinical endpoints than in unvaccinated Canadian WLWH, but higher rates than those seen in vaccinated women without HIV. Post-vaccination rates of persistent non-vaccine oncogenic HPV types were relatively high with a higher proportion of non-vaccine HPV types than of HPV types contained in the nonavalent vaccine.

**Conclusions:** These findings support the value of HPV vaccination and the need for ongoing cervical cancer screening post-vaccination in WLWH. They also do not indicate concern with

extending the spacing interval between the first and third doses up to two years. Collectively, these findings have provided great value to the clinical care of WLWH by informing best vaccination and screening practices for this particularly vulnerable population.

## LAY SUMMARY

Human papillomavirus (HPV) causes cervical cancer and genital warts. Women living with HIV (WLWH) experience higher rates of HPV infection, cervical cancer, and genital warts than women without HIV. Little was known about the HPV vaccine in WLWH. We addressed key aspects of HPV vaccination in WLWH using data from a cohort of WLWH in Canada. WLWH had high rates of HPV infection prior to vaccination, indicating strong need for the vaccine. Although recommendations are for three vaccine doses to be given within seven months, we showed that giving the doses within two years yielded a similar antibody response. Early assessment of the vaccine's ability to prevent infection and disease was promising but less robust than data from women without HIV. Post-vaccination, HPV types not contained in vaccines continue to infect these women. Collectively, these findings support the need for both HPV vaccination and continued cervical cancer screening in WLWH.

## PREFACE

This thesis is my original work and is based on the research conducted by myself in conjunction with my supervisor, Dr. Deborah Money, and the HPV in HIV Study Team. Ethical approval for central study coordination was obtained by the University of British Columbia Clinical Research Ethics Board with approval H08-00997. Additionally, all recruiting clinical sites received research ethics approval locally.

This research project as a whole would not have been possible without the contributions of many individuals. Pelvic exams were performed by health care professionals at each of the fourteen clinical sites. Cervical cytology samples were processed at the British Columbia Cancer Agency and HPV DNA samples were processed and typed by Dr. François Coutlée and his team at the Coutlée Laboratory in Montreal. My involvement in data collection was limited to the Oak Tree Clinic site in Vancouver, BC.

As this thesis describes data from a previously established cohort, study design was performed prior to the initiation of my role as a graduate student on the project. However, in consultation with study investigators, I selected the methods for analysis presented in the chapters of this thesis. I performed all statistical analyses presented herein with statistical support from Dr. Janet Raboud and Sandra Blitz at the University Health Network in Toronto, ON as well as Dr. Arianne Albert at the University of British Columbia in Vancouver, BC. I was also the first author and wrote first drafts, edited, and submitted all research chapters presented herein.

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| | |
|---|---|
| AGC: | Atypical glandular cells |
| ALP: | Alkaline phosphatase |
| ALT: | Alanine aminotransferase |
| ARV: | Antiretroviral |
| ASC-H: | Atypical squamous cells – cannot exclude HSIL |
| ASCUS: | Atypical squamous cells of undetermined significance |
| AST: | Aspartate aminotransferase |
| BUN: | Blood urea nitrogen |
| cART: | Combination antiretroviral therapy |
| CD4: | Cluster of differentiation 4 |
| CD8: | Cluster of differentiation 8 |
| CI: | Confidence interval |
| CIN1: | Cervical intraepithelial neoplasia grade 1 |
| CIN2: | Cervical intraepithelial neoplasia grade 2 |
| CIN3: | Cervical intraepithelial neoplasia grade 3 |
| cLIA: | Competitive Luminex immunoassay |
| CWHS: | Canadian Women's HIV Study |
| DNA: | Deoxyribonucleic acid |
| FDA: | Food and drug administration |
| GMT: | Geometric mean titre |
| HIC: | High-income country |
| HIV: | Human immunodeficiency virus |
| HPV: | Human papillomavirus |
| HSIL: | High-grade squamous intraepithelial lesion |
| ICC: | Invasive cervical cancer |
| IDU: | Injection drug use |
| IQR: | Interquartile range |
| IR: | Incidence rate |
| ITT: | Intention-to-treat |

| | |
|---|---|
| LSIL: | Low-grade squamous intraepithelial lesion |
| MHC: | Major histocompatibility complex |
| MTCT: | Mother-to-child transmission |
| NNRTI: | Non-nucleoside reverse transcriptase inhibitor |
| NRT: | Naïve to relevant type |
| OR: | Odds ratio |
| PCR: | Polymerase chain reaction |
| PI: | Protease inhibitor |
| PLWH: | Persons living with HIV |
| PPE: | Per-protocol efficacy |
| PY: | Person-years |
| qHPV: | Quadrivalent HPV (i.e., HPV6, 11, 16, 18) |
| RNA: | Ribonucleic acid |
| SCC: | Squamous cell carcinoma |
| VLP: | Virus-like particle |
| WBC: | White blood cell count |
| WHO: | World Health Organization |
| WLWH: | Women living with HIV |

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**DEDICATION**

*Til min bedstefar.*

*Jeg ville ønske, at du havde set dette.*

# CHAPTER 1: BACKGROUND

## 1.1 History of Histological Terminology for the Cervix

Terminology for HPV-associated lesion histology has changed many times over the past century as our understanding of disease development and availability of treatment strategies has changed. Beginning in approximately 1901, there was a single-tier system whereby someone could have or not have surface or intraepithelial carcinoma of the cervix (1). By the 1950's terminology had shifted to reflect the fact that lesions could be detected that did not represent carcinoma in situ; a four-tier system was put in place with terminology of mild, moderate, or severe dysplasia, or carcinoma in situ (2). In the early 1970's the term cervical intraepithelial neoplasia (CIN) was created to emphasize the fact that the grades of CIN (i.e. CIN1-3) were on a continuum leading to cervical cancer (3). Improved understanding of cervical oncogenesis and the acknowledgement of the challenges and lack of reproducibility in assigning a diagnosis of CIN2 (4, 5), led to the proposal of a two-tier system of low and high-grade intraepithelial lesions (6, 7). The two-tier system for histology was not supported by professional organizations at that time and was therefore not widely utilized, leaving the widespread use of the three-tier CIN1-3 system for histology in place into the 2000's.

Recent years have brought renewed support for a two-tier terminology system of high-grade and low-grade histology (low-grade squamous intraepithelial lesion – LSIL, includes CIN1; high-grade squamous intraepithelial lesion – HSIL, includes CIN2 and CIN3), which parallels the Bethesda System used for cervical cytology (8). This system better reflects the current state of knowledge about HPV-associated disease biology, it promotes better consistency between diagnoses, and it differentiates between diagnoses that

generally represent the threshold for treatment (CIN2 or higher). The primary argument against a two-tier system is that for adolescents and young women wishing to have future pregnancies, there is a much higher chance of CIN2 lesion regression and the harms of treatment are the greatest.

Due to the timing of the HPV in HIV Study described in this thesis, terminology used herein reflects the three-tier system of CIN1-3 that predominated for the majority of the active study years. In addition, many studies continue to utilize CIN3 as a study endpoint since it is a more reproducible diagnosis and a better indicator of a true premalignant lesion and surrogate for cancer risk.

### 1.2 Epidemiology of Cervical Cancer

Cervical cancer is responsible for a substantial burden of disease globally. There are over half a million new cases each year and over 300 000 deaths (9). In Canada, where cervix screening programs are well established, the burden of disease is due primarily to pre-invasive cervical disease. Despite this, over 500 deaths still occur annually in Canada due to cervical cancer (10). The burden of disease remains much higher in developing countries, which bear 83% of the yearly cases of cervical cancer due to challenges related to the implementation of screening programs in low resource settings (11). In sub-Saharan Africa, cervical cancer is the most common cancer and has a higher mortality rate than any other cancer in the region (9, 12).

Cervical cancer is caused by human papillomavirus (HPV) infection. This causal link has been well-established in the literature and is supported by the detection of HPV DNA in 96.6% of cervical cancer tissue (13). In addition to causing cervical cancer, HPV is also the

causal agent of genital warts and has been implicated in other genital tract cancers, head, neck, penile, and anal cancers (14). HPV is the most common sexually transmitted infection with an astounding lifetime risk of 75-80% (15). Risk factors for cervical cancer are tobacco use, early onset of sexual activity, multiple sexual partners, high-risk sexual partners, history of sexually transmitted infections, and history of vulvar or vaginal squamous intraepithelial lesions or cancer (15). Not surprisingly, all but one of these risk factors is associated with HPV acquisition risk.

More than 200 types of HPV have been identified and multiple types can concurrently infect an individual (16). The types are divided into high-risk oncogenic and low-risk non-oncogenic categories. The high-risk types are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68, 73, and 82 (17). The most frequently detected high-risk HPV types are 16 and 18, which account for 70% of cervical cancers worldwide (18).

HPV prevalence is highest among individuals aged 18-25. In Canada, the peak prevalence of HPV in women was found to be 20-25% in 20 year-old women (19, 20). Prevalence gradually decreases over time in ages greater than 25, sometimes reaching a plateau after age 40, and other times demonstrating slight increases in prevalence after age 40 (21). As a result of the high prevalence in ages 18-25 and the time it takes for oncogenesis to occur, cervical cancer prevalence is highest among women aged 30-59 (22).

A differential geographic distribution of HPV types is present whereby certain types are more common in different geographical locations. For example, HPV33 is most highly prevalent in Europe, while HPV52 and 58 are dominant in Asia, and HPV16, 58, 51, 66, and 18 are dominant in South Africa (23).

Depending on the HPV-infected cell type, cervical cancers can be squamous cell carcinomas affecting the squamous cells of the ectocervix, adenocarcinomas affecting the glandular cells of the endocervix, or a less common type of cervical cancer such as adenosquamous carcinoma, which affects both squamous and glandular cells.

### 1.3 Epidemiology of Genital Warts

As mentioned previously, HPV infection is also the causal agent of genital warts. The annual incidence of genital warts in Canadian provinces has been reported between 98-154 per 100 000, with higher rates seen in males than females (24). In the United States, genital warts are estimated to affect 1% of sexually active adults aged 15-49 with a similar annual incidence of approximately 100 per 100 000 (25, 26). Genital wart prevalence has been reported to be similar among immunocompetent individuals in other global settings. For example, a recent study in Nigeria found a genital wart prevalence of 1% in immunocompetent women (27).

Risk factors for genital warts mirror those for HPV infection since HPV is the cause. Therefore, risk factors include greater number of lifetime sexual partners, earlier sexual debut, smoking, and history of other sexually transmitted infections (26). Genital warts are often seen in the areas of friction in the anogenital region, due to the infectious process of HPV, whereby infectious virions require access to the basement membrane via breaks in the epithelium that may occur as a result of friction.

The most frequently detected HPV types contributing to the burden of genital warts are HPV6 and 11, which are responsible for approximately 90% of genital warts (28). While genital warts are not life threatening in nature, they are one of the most frequent sexually

transmitted infections, they can require painful treatment, and often result in a significant psychological and social burden (29, 30).

## 1.4 HPV Oncogenesis

### 1.4.1 HPV Infection and Oncogenic Process

HPVs are small, non-enveloped viruses with circular, double-stranded DNA. HPV infects basal cells of the stratified squamous epithelium, which it accesses through microlesions in the epithelium. It is critical that HPV infects basal cells as they are the only mitotically active cells of the epithelium. HPV initially binds to the basement membrane and then to heparin sulfate expressed on the basal cell surface via HPV's L1 capsid protein. The virus is internalized into the cell by a non-traditional form of endocytosis and is delivered to the cell nucleus (31). Once inside the nucleus, HPV completes a replication cycle, producing 50-100 viral genome copies, which stay in episomal form, as does the parent HPV genome (32). Infected basal cells have three possible division outcomes: 1) division into two non-dividing differentiated cells, 2) division into one parabasal and one basal cell, or 3) division into two basal cells which each have the ability to further divide (32). When an infected keratinocyte enters the differentiation compartment of the epithelium, viral DNA replication is activated such that infected cells will then contain many thousands of viral DNA copies per cell (33). Once the infected cells reach the outer epithelial layer and differentiation is complete, virions are formed and released as the differentiated cells perish and slough off (33).

Persistent HPV infection occurs due to the virus' ability to evade the immune system. The virus does not induce host cell lysis, the infection is very local in nature with an absence

of viremia, and infected cells at the superficial layer are programmed for apoptosis which does not produce danger signals the way that necrosis does (34). HPV oncogenes also suppress the production of interferon and decrease the attraction of antigen-presenting cells to the area of infection (35, 36). Five evolutionary genera of HPV exist: α, β, γ, μ, and ν. At least 60% similarity of L1 capsid protein is required to constitute HPV types being in the same genera. Genera differ in their modalities of immune system evasion; α-papillomaviruses employ a strategy of immune escape by suppressing the immune response through viral proteins while β-papillomaviruses minimize viral gene expression (32). The majority of high risk (oncogenic) HPVs are α-papillomaviruses as well as some low risk HPVs, including HPV6, 11, 16, and 18.

HPV has three functional regions in its genome: 1) the early region containing open reading frames, 2) the late region encoding structural proteins, and 3) the non-coding regulatory region. Early genes E5, E6, and E7 encode oncoproteins. E5 decreases the expression of major histocompatibility complex (MHC) I and II on the host cell surface, suppresses the antiviral interferon response, and enhances the activity of oncogenes E6 and E7 (37, 38). E6 induces p53 (tumour suppressor) degradation and cytokine production (39). E7 inhibits retinoblastoma protein activity (cell cycle regulators), disrupts DNA synthesis regulation, and stimulates progression of the cell cycle, resulting in genomic instability (32, 40). With high expression of oncoproteins, mutations may accumulate, cells lose their ability for apoptosis and growth suppression, and histological changes result. If left untreated, one third of cervical intraepithelial lesions of grade 2 and 3 will progress to cervical cancer (41). Eventually, cells may penetrate the basement membrane resulting in an invasive cervical cancer.

Integration of HPV into the host cell genome is seen in 10-12% of HPV infections and is seen in more than 70% of HPV-associated cancers (42, 43). As such, integration is seen to be an important, but not necessary, event in carcinogenesis. While integration impacts cell transformation, it is not a normal part of the viral life cycle and occurs due to the presence of microhomologous HPV sequences within the human genome (44). Following integration, the HPV DNA loses its infectivity and cannot be packaged into virions because a portion of the viral genome is lost during the process of integration. However, integration does not eliminate the activity of the E6 and E7 oncogenes, which continue to induce the oncogenic process (45). In the absence of integration, oncogenesis may still occur through a rare loss of growth control accompanying E6 and E7 activity, which keeps the cell cycle going such that viral replication may continue (33).

### 1.4.2. Screening Modalities for HPV and Precancer

Since the 1950s, cervical cancer screening by Papanicolaou's smear (i.e., cervical cytology) has been endorsed in Canada. In fact, British Columbia, Canada was the first jurisdiction globally to implement a cervical cancer screening program, led by Dr. David Boyes (46). Cervical cytology involves the collection of cervical cells, which are spread onto a slide and analyzed for any abnormality that is consistent with precancerous changes. This allows for the earlier detection of these changes, which have no symptoms and would otherwise go unnoticed. Treatment of precancerous changes prevents invasive cervical cancer and its associated morbidity and mortality.

Adoption of programmatic cervical cytology screening in Canada has resulted in a 50% decrease in the incidence of cervical cancer and a 70% decrease in the mortality of

cervical cancer between 1969-1992 (46, 47). Although cervical cytology has had a substantial impact on prevention of cervical cancer, it is limited in some important ways. Women must adhere to screening recommendations and undergo screening on a regular basis. Inadequate screening is present in approximately 50% of cervical cancer cases (48). Other challenges include false negatives, inadequate sampling of the cervical tissue, and inadequate follow-up of abnormal results (47).

In recent decades, the understanding of HPV as the causal agent of the vast majority of cervical cancers has led to the development of HPV testing as a screening modality. HPV testing is completed by collection of a cervico-vaginal sample on a swab and HPV testing on the sample using an HPV assay. By detecting high risk HPV DNA, this test is able to identify women with an infection to allow diagnosis of those who may have associated dysplastic changes. It is much more sensitive than cervical cytology for detection of precancerous changes, allowing for earlier treatment (49, 50). A number of HPV assays have now been Health Canada approved and/or FDA-cleared for use alongside cervical cytology (i.e., co-testing) or as a primary screening method. In contrast to cervical cytology, HPV assays do not require a sample of cells from the transformation zone so a woman may collect the sample herself, which may increase acceptability of this screening method in many global settings.

## 1.5 HPV in the Context of HIV Infection

### 1.5.1 Basic Interaction

Since the host immune response is critical to clear HPV infection, the effects of HIV on the immune system are thought to increase the pathogenesis of HPV in women living with

HIV (WLWH). Supporting this, low CD4+ T cell counts are correlated with high risk HPV infection (51). WLWH have 4-fold higher rates of HPV infection than women without HIV (52). An American study found that by eight years of follow-up, 92% of their cohort of WLWH had experienced detectable HPV infection (53).

Mechanisms by which HIV may facilitate infection with HPV have been proposed including the disruption of epithelial tight junctions by cellular secretions due to HIV infection and effects relating to the immune dysfunction at the mucosal site that accompanies HIV infection (54, 55). Compromised T cell functionality, and thus compromised cell-mediated immunity, plays a significant role in the increased pathogenicity of HPV in WLWH due to their reduced ability to resolve pre-malignant lesions (56). This reduced ability to clear existing HPV infection can be somewhat mitigated by the use of combination antiretroviral therapy (57). However, it is important to note that due to the advent of combination antiretroviral therapy, WLWH are living longer lives and therefore more frequently developing dysplasia and cervical cancer. These unique circumstances in WLWH make prevention of HPV infection particularly important within populations living with HIV.

### 1.5.2 Cervical Cancer in Women Living with HIV

Due to higher rates of HPV infection combined with effects of HIV infection (i.e., reduced CD4 counts and presence of HIV viremia), WLWH experience up to seven-fold higher rates of the resultant cervical dysplasia and cancers than women without HIV, and HIV-positivity is in fact predictive of high-grade squamous intraepithelial lesions (HSIL) (58, 59). The standardized incidence ratio of cervical cancer in WLWH was found to be 5.82 in meta-analysis indicating excess cases of almost 600% over the general population (60).

A high prevalence of uncommon HPV types has been documented in WLWH, which may call for screening and prevention methods that are specific to this population (52, 61). Additionally, infection with multiple types of HPV is more common in WLWH and HPV infection tends to be more persistent, allowing for the more frequent and rapid progression to cervical dysplasia and invasive cancer (62-65). The situation in which WLWH are severely immunocompromised is particularly dire, as demonstrated by findings of 20% of an immunocompromised cohort having squamous cell carcinoma (SCC) of the cervix and 33% within the same cohort having cervical HSIL. Overall, 94% of the cross-sectional cohort had abnormal cervical cytology (66).

### 1.5.3 Genital Warts in Women Living with HIV

Just as rates of cervical cancer are higher among WLWH, so too are rates of genital warts (67). The prevalence of genital warts among WLWH in Nigeria was recently found to be five times greater than the prevalence among women without HIV. Additionally, the incidence of genital warts over the study period was almost three times greater at 1370 per 100 000 person-years (27). Similarly, in the United States, genital wart prevalence was approximately three times greater among WLWH (68). Persons living with HIV (PLWH) may have larger warts, larger clusters of warts, and may not respond as well to treatment, with a higher rate of recurrence (68, 69).

### 1.5.4 HPV and HIV in Indigenous Girls and Women

A population particularly affected by both HPV and HIV in Canada and globally is Indigenous girls and women. In numerous countries globally, higher rates of HIV infection

are seen in Indigenous people, compared to non-Indigenous people (70). Canada is no exception; although Indigenous people comprise only 4.9% of the Canadian population, they represent 10% of people living with HIV and 11% of new HIV infections in Canada (71). Indigenous women also experience higher rates of cervical cancer incidence and mortality than their non-Indigenous counterparts (72). In Canada, cervical cancer incidence is almost 3.5 times greater among Indigenous women while cervical cancer mortality is 4 times greater (73). This increased cervical cancer incidence is due to higher rates of HPV infection coupled with lower rates of cervical cancer screening (74-76). Strategies to prevent HPV infection, such as HPV vaccination, and increase screening uptake, such as HPV self-sampling, will be particularly important for Indigenous girls and women (77).

## 1.6 HPV Vaccination

### 1.6.1 HPV Vaccine Basics

HPV vaccines are currently available in bivalent and nonavalent formulations, and previously in a quadrivalent formulation. The bivalent vaccine protects against HPV types 16 and 18 thus offering protection against 70% of cervical cancers. The quadrivalent vaccine protected against types 6, 11, 16, and 18 thus protecting against 70% of cervical cancers and 90% of genital warts and was available from 2006 to 2017. The nonavalent vaccine, which was licensed in 2014 and incorporated into public health programs shortly thereafter, protects against five additional HPV types (31, 33, 45, 52, and 58), thereby offering protection against up to approximately 90% of cervical cancers. The vaccines differ in not only the HPV types, but also in the adjuvant used. While the bivalent vaccine contains an ASO4 adjuvant, the quadrivalent and nonavalent vaccines contain a proprietary alum adjuvant (78-80).

The vaccines are composed of virus-like particles that utilize the L1 capsid protein to self-assemble into the outer shell of a virion while not containing any viral DNA. Exposure to the vaccine produces high levels of circulating, systemic anti-HPV neutralizing antibodies. Protection from incident HPV infection must occur locally, which is achieved through transudation of systemic antibodies into the cervix or exudation of antibodies to a micro trauma of the vagina or vulva (81).

Initial vaccine schedules recommended three doses of vaccine at month zero, one or two, and six (82). However, more recent data has suggested that among people aged 9-14, two doses are non-inferior in terms of antibody response generated in immunocompetent recipients (83). In fact, some data suggest that one dose may produce non-inferior efficacy (84). Due to the timing of the study described herein (commencing prior to any one or two-dose data), and the current recommendation of three doses for all girls and WLWH (85, 86), participants described in this thesis were scheduled to receive three doses of vaccine.

### 1.6.2 HPV Vaccine Safety and Immunogenicity

The HPV vaccines have demonstrated exceptional safety profiles in immunocompetent girls and women and are highly immunogenic. The most commonly reported adverse effect of the vaccine is pain at the injection site. Rates of serious adverse events did not differ significantly between individuals receiving the bivalent vaccine, quadrivalent vaccine, or placebo (87). Licensure studies on the quadrivalent vaccine demonstrated seroconversion in 99-100% of subjects at month 7 with geometric mean titers (GMTs) reduced at 36 months but still conferring protection (88, 89). Anti-HPV16 GMTs for all vaccines reach a plateau phase that is well above the level conferred by natural infection

and this plateau is sustained over at least 10 years (90). Anti-HPV18 GMTs are not as well sustained and drop to levels similar to those achieved with natural infection by as early as two years from vaccination; at 9 years of follow-up, only 60% of per-protocol participants remained seropositive to HPV18 on the competitive Luminex immunoassay (90). Whether this reduction in HPV18 seropositivity will result in breakthrough HPV18-associated disease is not yet clear, but evidence to date in persons without HIV suggests that HPV18-associated disease is not occurring despite reduced seropositivity (90).

### 1.6.3 HPV Vaccine Efficacy

HPV vaccine efficacy was exceptional in licensure studies. Efficacies in per protocol populations were consistently above 90% while efficacies in intention-to-treat populations varied dramatically depending on analysis strategies employed, ranging from 44% efficacy against HPV16/18-associated CIN2+ in the FUTURE II trial to 93% efficacy against qHPV-associated infection and disease in another multinational study (88, 91-93). The wide range of intention-to-treat efficacies can be accounted for by the analysis strategies employed, whereby studies had differing inclusion criteria (age range, percentage of women with pre-existing infection or disease), case counting strategies (when to begin counting cases post-vaccination), or duration of follow-up (with extended follow-up, pre-existing cases in the intention-to-treat group are exhausted while additional cases continue to accrue in the placebo group). Ecological data have now demonstrated the high efficacy of HPV vaccination programs in countries that have implemented them. In countries with greater than 50% vaccination coverage among females, HPV16 and 18 infections decreased by 68% and genital warts decreased by 61% in girls aged 13-19 (94). Data linkage from British Columbia

has demonstrated a vaccine efficacy against cervical intraepithelial lesions of grade 2 or higher of almost 60% (95).

## 1.7 Vaccination in Individuals Living with HIV

### 1.7.1 Vaccination Efficacy in Individuals Living with HIV

Not only is cellular immunity impaired with HIV infection, but humoral immunity is impacted as well. As vaccines rely upon the recipient's immune system to generate protection, vaccines have generally been shown to elicit a reduced response in PLWH compared to the general population (96, 97). For example, some vaccines with reduced immunogenicity and efficacy in PLWH include hepatitis A, hepatitis B, tetanus, diphtheria, and polio vaccines (96, 97). Some studies have documented improved vaccine responses in PLWH who have higher CD4+ T cell counts (97-99), suggesting that HIV treatment can improve vaccine response. Additionally, the duration of seroprotection among PLWH tends to be shorter than among persons without HIV, which suggests a need for booster doses (100).

### 1.7.2 HPV Vaccination in Individuals Living with HIV

Despite reduced immunogenicity and efficacy of some other vaccines in PLWH, the existing data on HPV vaccination among PLWH has been promising. The HPV vaccine has been found to be safe and immunogenic in PLWH (101-104). Kojic et al. found that seroconversion at week 28 among WLWH was between 75%-100% depending on the HPV type and CD4+ T cell count of the participants; the lowest rate of seroconversion was to HPV18, as seen in HIV-negative populations, and among women with a CD4 count below

200 cells/μL. It appears that the use of antiretrovirals may assist the immunogenicity of the vaccine in this population. Participants taking antiretrovirals experienced 100% seroconversion to quadrivalent HPV types while those not on antiretrovirals showed rates of 92-100% seroconversion (103). Similarly, data from the study presented herein demonstrated increased immunogenicity among WLWH who had suppressed HIV viral loads (105). Seroconversion rates in our population of WLWH were between 94-99% (105).

Aside from the data presented in this thesis, there remains no published data on the efficacy of the HPV vaccine in preventing cervical dysplasia and cancer among WLWH.

### 1.8 Global Elimination of Cervical Cancer

In 2018, the World Health Organization (WHO) announced a global call to action towards elimination of cervical cancer (i.e. <4 cases/100,000 women-years) (106). The WHO will release its draft strategy in May 2020 (106) and Canada has committed to supporting this call to action (107). Given the burden of cervical cancer, achievement of the WHO's ambitious elimination goal will require new approaches and deeper understanding of at-risk populations, including women living with HIV. School-based HPV vaccination programs alone will lead, optimistically, to elimination of cervical cancer at the end of the 21$^{st}$ century (108). More rapid elimination will require scale up of screening and treatment and vaccination programs that go beyond the school-based model to target additional age groups and key populations (109, 110). Importantly, HIV is a major global contributor to HPV incidence and cervical cancer development, resulting in significantly higher rates of disease in the world's 17.8 million WLWH (111). Therefore, global elimination strategies must include an evidence-based plan to immunize WLWH prior to sexual debut, across high-risk

periods for HPV acquisition, and into adulthood. The study presented in this thesis was uniquely able to provide novel analyses on aspects of HPV vaccination in WLWH due to the presence of the established national cohort in a country with access to HPV vaccination. Although these analyses will later need to be corroborated with further evidence from other countries, they provide critical early findings to inform the strategy for global elimination of cervical cancer.

# CHAPTER 2: RATIONALE AND OBJECTIVES

## 2.1 Rationale

Women living with HIV are among the most vulnerable people globally to HPV infection and HPV-associated disease. As such, primary prevention by HPV vaccination is of utmost importance for this population. The presence of geographic diversity in HPV type prevalence requires that continent, and often country-specific, data on HPV prevalence is needed. Additionally, prevalence of HPV types can change over time and is impacted by the introduction of vaccine programs; following implementation of HPV vaccination programs, the burden of vaccine HPV types is reduced (112, 113). Data on HPV type prevalence among women living with HIV in Canada has become outdated and has not been reported for data beyond the early 2000's (114).

The HPV vaccine has a strong immunogenicity and efficacy profile in immunocompetent populations. At the initiation of this thesis work, there was no published data on HPV vaccine efficacy in women living with HIV. Although some immunogenicity data from women living with HIV were published, in the absence of a known immune correlate of protection for HPV, efficacy data is truly critical to inform the utility of the HPV vaccine in women living with HIV.

The next step beyond describing HPV vaccine efficacy is to utilize this and other data to inform HPV vaccination and HPV screening policies. Following HPV vaccination with the quadrivalent vaccine, the oncogenic HPV types that contribute to persistent HPV infection in women living with HIV still pose a risk for cervical precancer and cancer development. Given that women living with HIV experience a wider range of oncogenic HPV type

infections (64), an assessment of oncogenic HPV types persistently infecting women living with HIV who have been vaccinated with the quadrivalent vaccine will inform the need for higher vaccine valency in this population. This data will also inform guidelines that involve HPV testing as a cervical screening modality.

Data assessing the impact of HPV vaccine dose number and spacing of vaccine doses can also have a substantial impact on HPV vaccination programs for women living with HIV. The timing and number of HPV vaccine doses in girls and young women without HIV has changed over time as additional data have supported reduced dose schedules and demonstrated non-inferiority of wider intervals between doses. Reduced dose schedules pose significant cost-savings that may make HPV vaccination programs financially feasible in some global settings where three-dose schedules are not (115). In addition, data that explore the impact of wider vaccine dose spacing beyond the recommended 0, 1/2, and 6 months can inform programs for women living with HIV with respect to the importance of adherence to this timeline. If there is a detrimental impact of wider vaccine dose spacing, there may be a need for an additional booster dose or re-initiation of the vaccine schedule in women who do not receive their doses at the recommended time. Conversely, if there is not a significant reduction in vaccine immunogenicity with wider spacing of vaccine doses, vaccine providers will not need to administer additional doses, resulting in programmatic cost savings.

Globally, there are 19.6 million girls and women living with HIV (116). Research on the HPV vaccine in this sizable population of females that is particularly vulnerable to HPV will have a substantial impact on achieving the global goal of cervical cancer elimination (106). Although the data presented in this thesis was generated in a cohort of WLWH in Canada, the impact of our findings are of great importance for other global settings where the

intersection of HIV as an endemic, little or no HPV vaccine availability to date, and little or no cervical screening results in the highest rates of invasive cervical cancer and cervical cancer mortality globally. Despite important differences between Canada and global settings with high HIV seroprevalence, data to inform the utility of HPV vaccination in WLWH in Canada will provide evidence that can inform HPV vaccination programs for other WLWH worldwide.

### 2.2 Objectives

Due to the paucity of available data on HPV vaccination in girls and women living with HIV, the purpose of this thesis is to examine the overall impact of the HPV vaccine in girls and women living with HIV across Canada. This purpose is achieved by the following four objectives:

1. To assess the prevalence, persistence, and predictors of oncogenic HPV infection prior to vaccination in a cohort of women living with HIV in Canada.
2. To assess the effect of differential dose spacing of the quadrivalent HPV vaccine on vaccine immunogenicity in a cohort of women living with HIV in Canada.
3. To assess the 2-year efficacy of the quadrivalent HPV vaccine in a cohort of women living with HIV in Canada by determining rates of persistent HPV infection, genital warts, and cervical intraepithelial neoplasia of grade 2 or higher and comparing to published literature from women without HIV.
4. To assess rates of new persistent infection with oncogenic HPV types not contained in the quadrivalent HPV vaccine in our cohort of quadrivalent HPV-vaccinated women living with HIV in Canada.

## 2.3 Contents of the Thesis

This thesis is manuscript-based and addresses the objectives described above in eight chapters. Chapter 1 provides background on the epidemiology of HPV and HPV-associated disease, the biological basis of HPV oncogenesis, the interaction between HPV and HIV, HPV vaccination in the general population, and vaccination, HPV and otherwise, in persons living with HIV.

Chapter 2 describes the rationale, four key objectives, and contents of the thesis.

Chapter 3 describes the overall methods of the HPV in HIV Study. This national study has generated the dataset from which all analyses within the thesis are produced. Further details of methodology pertaining to each analysis are provided in the respective thesis chapter of the analysis.

Chapters 4-7 are research chapters that investigate various aspects of HPV vaccination in girls and women living with HIV. Chapter 4, Prevalent and Persistent Oncogenic HPV Types in a Cohort of Women Living with HIV Prior to HPV Vaccination, in revisions in 2020, provides a description of the prevalence, persistence, and predictors of oncogenic HPV infection in women living with HIV in Canada prior to HPV vaccination. It also describes cases of high-grade cervical cytology and the associated HPV types in this population. Chapter 5, Immunological Impact of Quadrivalent HPV Vaccine Dose Spacing in Women Living with HIV, published in *Vaccine* in 2020 (117), assesses the impact of differential dose spacing of the quadrivalent HPV vaccine on vaccine immunogenicity in women living with HIV. Chapter 6, The Efficacy of the Quadrivalent Human Papillomavirus Vaccine in Girls and Women Living with HIV, published in *Clinical Infectious Diseases* in

2019 (118), provides the first HPV vaccine efficacy data in women living with HIV. Chapter 7, Persistence of Non-Vaccine Oncogenic HPV Genotypes in Quadrivalent HPV-Vaccinated Women Living with HIV, published in the *Journal of Acquired Immune Deficiency Syndromes* in 2020, assesses the rates of persistent infection with oncogenic HPV types not contained in the quadrivalent HPV vaccine in quadrivalent HPV-vaccinated women living with HIV in Canada.

Chapter 8 concludes the thesis with a summary of the thesis findings, their contribution, and future directions for research in this area.

# CHAPTER 3: METHODS OF THE HPV IN HIV STUDY

## 3.1 Study Design

"A Study of an HPV VLP Vaccine in a Cohort of HIV Positive Girls and Women" and "Long-term Follow-up Study of CTN 236 – A Study of an HPV VLP Vaccine in a Cohort of HIV Positive Girls and Women," collectively and informally known as the HPV in HIV Study, is a prospective, longitudinal cohort study. The Women's Health Research Institute at BC Women's Hospital and Health Centre is the study-coordinating centre and the location of the Oak Tree Clinic. An additional thirteen sites providing care to girls and women living with HIV across Canada have also participated in the study. The primary objective of the base and long-term follow-up studies was to evaluate seroresponsiveness to the quadrivalent HPV vaccine and to measure antibody response to each of the vaccine genotypes out to 96 months post vaccination in girls and women living with HIV.

Due to the fact that the vaccine was licensed and promoted for use in females with HIV, despite a lack of data, at the time of study initiation, it was deemed to not be ethical to conduct the study as a placebo-controlled trial. Therefore, this study offered quadrivalent HPV vaccine in a three-dose schedule to all participants in a longitudinal single cohort design, as this was the most feasible design to provide valuable data on this topic. The lack of a placebo group is mitigated by the fact that extensive published literature from licensure trials, including Canadian sites accessing comparable populations, exists in girls and women without HIV with which comparisons may be made. In order to ensure comparability with published data on the quadrivalent HPV vaccine, serology was provided in-kind by Merck on

the proprietary competitive Luminex immunoassay (cLIA) and all doses of vaccine were also provided in kind by Merck.

### 3.2 Study Population

Girls and women living with HIV, aged 9 and older, attending study centres across Canada, were eligible for inclusion in this study. Additional inclusion criteria were: able to provide informed consent or assent, not pregnant and willing to avoid pregnancy throughout the vaccination period, able to attend clinic for the study visits, and having a cervix. Potential participants were ineligible if they were allergic to any of the vaccine components, had already received any doses of the HPV vaccine, were currently enrolled in a trial of an investigational vaccine or drug, or had any condition that the site investigator deemed exclusionary (e.g. poor prognosis or extreme immunocompromise).

Four hundred and twenty girls and women receiving care at clinics in British Columbia, Ontario, and Quebec were enrolled in the base study, with 353 females receiving at least one dose of vaccine. Two hundred and forty-one girls and women were re-engaged in the long-term follow-up study. Baseline demographics of all participants who received at least one dose of vaccine (n=353) are described in Table 1.1. The population is further described in early publications of the study (105, 119).

**Table 1.1** Baseline Demographics of the HPV in HIV Study Cohort (n=353)

| Characteristic | Median (IQR) or N (%) |
|---|---|
| Baseline age (years) | 36 (27-43) |
| Region of origin | |
| Africa | 122 (34.6%) |
| Asia | 13 (3.7%) |

| | |
|---|---|
| Canada | 180 (51.0%) |
| Caribbean | 20 (5.7%) |
| Central America | 6 (1.7%) |
| Europe | 6 (1.7%) |
| Middle East | 1 (0.3%) |
| South America | 5 (1.4%) |
| Ethnicity | |
| Asian | 21 (5.9%) |
| Black | 169 (47.9%) |
| Hispanic | 5 (1.4%) |
| Indigenous | 42 (11.9%) |
| White | 112 (31.7%) |
| Other | 4 (1.1%) |
| Probable mode of HIV acquisition[+] | |
| IV drug use | 47 (13.3%) |
| Sexual contact | 221 (62.6%) |
| Mother-to-child transmission | 63 (17.8%) |
| Blood products | 18 (5.1%) |
| Other | 31 (8.8%) |
| Baseline CD4 count (cells/$mm^3$) | 523 (384-710) |
| Baseline CD4 nadir (cells/$mm^3$) | 242 (123-367) |
| Baseline antiretroviral therapy | |
| PI based | 165 (46.7%) |
| NNRTI based | 95 (26.9%) |
| Other | 31 (8.8%) |
| Previously on therapy | 14 (4.0%) |
| Not started | 22 (6.2%) |
| Unknown | 17 (4.8%) |
| Baseline HIV viral load suppressed | |
| Yes | 236 (66.9%) |
| No | 96 (27.2%) |
| Unknown | 21 (5.9%) |
| Number of vaccine doses | |
| 1 | 20 (5.7%) |
| 2 | 15 (4.2%) |
| 3 | 318 (90.1%) |

[+]Participants could indicate more than one probable mode of HIV acquisition, therefore, percentages do not add to 100%

### 3.3 Condensed Study Protocol

Participants were scheduled to complete eight visits in the base study (a screening visit at -3 months, a baseline visit at time zero, and follow-up visits at months 2, 6, 7, 12, 18, and 24) and three visits in the long-term follow-up study (Table 1.2). Due to the nature of enrollment and vaccination occurring over the span of multiple years with a delay between the base and long-term follow-up studies, some participants were eligible for the month 36 visit, while others were further out from vaccination and may have only been eligible for months 72, 84, and 96, for example.

**Table 1.2** Study Visit Schedule and Procedures

| | Comprehensive Medical History | HPV Vaccine | Review of Symptom Diaries | Physical Exam | βHCG Pregnancy Test | Blood Work (Clinical & Serology) | Pelvic Exam | Cervical Cytology & HPV DNA |
|---|---|---|---|---|---|---|---|---|
| Screening (-3 months) | X | | | X | X | X | X | X |
| Visit 1 (Baseline) | | X | X | X | X | X | X | X |
| Visit 2 (2 months) | | X | X | X | X | X | | |
| Visit 3 (6 months) | | X | X | X | X | | X | X |
| Visit 4 (7 months) | | | X | X | | X | | |
| Visit 5 (12 months) | | | | X | | X | X | X |
| Visit 6 (18 months) | | | | X | | X | X | X |
| Visit 7 (24 months) | | | | X | | X | X | X |
| Visit 8 (36 months) | | | | X | | X | X | X |
| Visit 9 (48 months) | | | | X | | X | X | X |
| Visit 10 (60 months) | | | | X | | X | X | X |
| Visit 11 (72 months) | | | | X | | X | X | X |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Visit 12 (84 months) | | | | X | | X | X | X |
| Visit 13 (96 months) | | | | X | | X | X | X |

At the screening visit, consent/assent was obtained prior to any study procedures. Following the consent process, participants provided a comprehensive medical history, blood for clinical and study investigations, and a pregnancy test. The physician also conducted a physical and pelvic exam, including cervical cytology and HPV DNA testing. In the case where participants were pre-menarchal and not sexually active, they did not undergo pelvic examination until clinically appropriate, at the discretion of the site investigator.

Visits one, two, and three (i.e., baseline, month 2, and month 6) were vaccination visits where participants received the quadrivalent HPV vaccine intramuscularly. Prior to vaccination at each of these visits, participants underwent a pregnancy test, physical exam, and additional study sampling as per Table 1.2. Following vaccination, participants were monitored for 30 minutes and were called after 48 hours to review any serious adverse events. Participants also received a symptom diary to record their post-vaccination symptoms up to 30 days after each dose as part of the safety monitoring process.

A limited visit was performed during visit four (i.e., month 7) where a symptom diary review and physical exam were completed alongside blood work for serology. All following visits included physical examination, blood work, and pelvic examination including cervical cytology and HPV DNA testing. Ongoing consent/assent was obtained at all visits.

## 3.4 Study Samples

### 3.4.1 Clinical Blood Work

Results from the following clinical blood work, if conducted within four weeks of a study visit, was documented as part of the study: hemoglobin, WBC, platelets, ALT, AST, ALP, bilirubin, BUN, serum creatinine, CD4, CD8, and HIV RNA quantitative PCR.

### 3.4.2 Study Blood Work (HPV Serotesting)

Venous blood was collected in a 10 mL red-top Serum Vacutainer. Samples were allowed to clot and then refrigerated until centrifuged. Serum was then aliquotted into four cryovials of a minimum 1 mL and frozen at -80 degrees Celsius. Samples were shipped frozen to Merck Laboratories where serology was performed by competitive Luminex immunoassay (cLIA). The cLIA quantifies the antibodies present to a single neutralizing epitope for each of the four HPV types present in the vaccine through the emission of light or lack thereof upon binding of antibodies in human serum to virus-like particles after they have outcompeted the pre-bound monoclonal antibodies. Results of cLIA are provided in milli-Merck units per mL, which can be interpreted as an antibody concentration. Due to the fact that binding affinities differ between the different monoclonal antibodies used, one cannot directly compare the antibody titers generated for each of the four HPV types.

### 3.4.3 Cervical Cytology

Cervical cytology was performed using the ThinPrep Pap Test, a liquid-based cytology test. In the context of a speculum examination, cervical cells were obtained using a broom-shaped endocervical cytobrush. The brush was then rinsed in PreservCyt solution and

the sample of solution was kept at ambient temperature. All sites shipped their cervical samples to the BC Centre for Disease Control where the samples were processed and sent on to the Cervical Cancer Screening Laboratory of BC for interpretation. At the Cervical Cancer Screening Laboratory of BC, samples were read by cytotechnologists with standard reporting according to the Bethesda criteria (6).

### 3.4.4 HPV DNA Testing

An aliquot of the PreservCyt solution received by the BC Centre for Disease Control was sent for centralized HPV DNA testing at the Coutlée Laboratory in Montreal, Canada. HPV DNA testing was performed by Linear array assay (Roche Molecular Systems). The test first amplifies HPV DNA by polymerase chain reaction, which is then reverse hybridized onto a nylon strip and typed. A positive/negative result is produced for 36 different types of high and low-risk HPV: types 6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, and 89. All sample runs were accompanied by positive and negative controls.

# CHAPTER 4: PREVALENT AND PERSISTENT ONCOGENIC HPV TYPES IN A COHORT OF WOMEN LIVING WITH HIV PRIOR TO HPV VACCINATION

## 4.1 Introduction

Cervical cancer remains a leading cause of mortality for women throughout the world due to lack of comprehensive screening and treatment, and limited implementation of vaccine programs globally (120, 121). Women living with HIV (WLWH) experience approximately double the prevalence of HPV infection compared to their HIV-negative counterparts (53, 114). In addition, WLWH are more likely to experience infection with less common HPV types, concurrent infections with multiple types, and persistent infection (62, 64). Collectively, these factors contribute to the higher rates of cervical cancer in WLWH, compared to women without HIV. Data from North America has shown that the incidence rate of cervical cancer among women without HIV is 5 per 100 000 person-years, while in WLWH it is 16 per 100 000 person-years (122).

Three prophylactic HPV vaccines have been used in vaccination programs over the last decade. The bivalent vaccine (Cervarix®) provides protection against HPV16/18 and the quadrivalent vaccine (GARDASIL™) protects against HPV6/11/16/18. A nonavalent HPV vaccine (GARDASIL®9) was more recently licensed and protects against HPV6/11/16/18/31/33/45/52/58. While these vaccines have proven safe and highly efficacious in HIV-negative populations (88, 91, 92), their efficacy in individuals living with HIV is less well established.

When making public health decisions regarding which vaccine is ideal for WLWH and HPV type-based triage in HPV-based cervical screening programs, it is crucial to

consider the type-specific HPV prevalence in populations living with HIV. With more comprehensive and successful management of HIV and the introduction of HPV vaccines into routine use, existing data on HPV types in persons living with HIV may be outdated (114, 123, 124). It is consequently critical to understand the current burden of oncogenic HPV infection in WLWH who are engaged in care and accessing combination antiretroviral therapy. In this analysis, we assessed the prevalence, persistence, and predictors of oncogenic HPV infection prior to vaccination and determined rates of high-grade cervical cytology and its relationship to specific HPV types in a cohort of WLWH in Canada.

**4.2 Methods**

Girls and women living with HIV (WLWH), aged 9 and greater, were recruited into a prospective study of HPV vaccination from 14 sites of HIV care across Canada. As there was no maximum age for this study, the vaccine was used beyond the age limit of FDA approval. The study and methods have been previously published (105). In brief, participants were ineligible if they had received any prior doses of HPV vaccine, had an allergy to vaccine components, were currently enrolled in another study of an investigational drug or vaccine, or if a site investigator deemed their health to be exclusionary. Ethical approval for the coordination of this study was received from the University of British Columbia Clinical Research Ethics Board (H08-00997) and each clinical recruitment site received local ethics approval.

The primary objective of the overall study was to assess the immunogenicity and efficacy of the quadrivalent HPV vaccine in WLWH (105, 118); secondary objectives

included a planned assessment of the pre-vaccination oncogenic HPV infection and cervical cytology.

For this analysis we utilized the screening and baseline study visits, planned at three month intervals but with an actual median of 4 months apart (range: 3-22 months). At both of these study visits, participants underwent a clinical assessment, a pregnancy test, study blood work, cervical cytology, and HPV DNA sampling. Cervical cytology samples utilized liquid-based cytology. These cytology samples were processed by a single reference laboratory at the British Columbia Cancer Agency and were reported using Bethesda criteria. Aliquots of the cytology samples were sent to a single laboratory for HPV DNA testing using the Linear array assay to provide a positive or negative result for 36 types of HPV as described previously (125). At the end of the baseline visit, participants received their first dose of quadrivalent HPV vaccine.

The objectives of this analysis were to assess cervical cytology results and their relationship to oncogenic HPV types detected in our cohort, to determine predictors of type-specific HPV persistence between two visits, and to determine the attributable HPV types in cases of high-grade cervical cytology. All statistical analyses were performed in R (version 3.2.2). Oncogenic HPVs were divided into categories based on their presence in currently available HPV vaccines. Logistic regression was utilized for both univariate and multivariate analysis. All variables that were significant upon univariate analysis were included in the multivariate model.

### 4.3 Results

420 participants were enrolled in the study, of which 252 were eligible for this analysis (Table 4.1). Participants were eligible if they had two pre-vaccination HPV DNA results that were at least 3 months apart and at least one pre-vaccination cervical cytology result. The median age was 39 years (IQR: 33-45, range: 16-65) with mixed ethnicity: predominantly Black (111 [44.0%]) and White (91 [36.1%]) ethnicities, 32 (12.7%) Indigenous, and 18 (7.1%) other. The median number of lifetime sexual partners was 6 (IQR: 3-12). The most frequently self-reported mode of HIV acquisition was sexual contact (187 [74.2%]). In terms of antiretroviral use, 217 (86.1%) of participants were on a regimen, 19 (7.5%) were not on therapy, and 16 (6.3%) had unknown antiretroviral use status. Of those on antiretrovirals, 123 (56.7%) were on protease inhibitor (PI)-based regimens and 66 (30.4%) on non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens. The median CD4 count at baseline was 510 cells/$mm^3$ (IQR: 388-698, range: 11-1610) and the median CD4 nadir was 240 cells/$mm^3$ (IQR: 111-340, range: 0-1078). One hundred and eighty (72.9%) of 247 participants with HIV viral loads available were HIV virologically suppressed (<50 copies/mL) at baseline. Thirty-eight (16.1%) of 236 participants with hepatitis C results available were co-infected with hepatitis C virus.

At the baseline visit, HPV16 and HPV52 were the most prevalent oncogenic HPV types (10.3% and 9.1%, respectively) (Table 4.2). The next most prevalent types, in order of most to least prevalent, were HPV45, 51, 56, 58, 59, and 18. HPV16, 52, and 45 were also the most frequent types associated with persistent infection between the screening and baseline visits (7.5%, 6.3%, and 6.3%, respectively). The additional HPV types contributing to persistent infection, in order of most to least frequent, were HPV56, 58, 35, 39, 18, 51, and 59. Overall, almost half of the population (45.2%) was infected with at least one oncogenic

HPV type at baseline and 33.3% of participants had a persistent oncogenic infection between the two study visits.

Oncogenic HPV types were also divided into categories based on their presence in available vaccines. These categories were: HPV16, HPV18, additional nonavalent types (HPV31/33/45/52/58), and other high-risk types (HPV35/39/51/56/59/68/82) which are not protected against by any vaccine (Table 4.3). 7.5% of participants had a persistent HPV16 infection, 3.2% had a persistent HPV18 infection, 17.9% had a persistent infection with at least one of the additional nonavalent HPV types (HPV31/33/45/52/58), and 17.1% had persistent infections with at least one oncogenic HPV type not contained in any currently available vaccine (HPV35/39/51/56/59/68/82).

Hypothesis testing was not performed for HPV18 due to the low number of cases. Univariate analyses of potential predictors of HPV persistence demonstrated no significant difference between women with and without persistent oncogenic HPV infection in demographic variables (including age, ethnicity, lifetime sexual partners, and region of origin) or hepatitis C status, aside from lower age being associated with HPV16 persistence (p=0.04, OR=0.95 [95% CI: 0.89-0.99]) (Table 4.4). Women with persistent HPV16 and persistent other high-risk HPV were more likely to have an unsuppressed HIV viral load of >50 copies/mL (p=0.02, OR=3.2 [95% CI: 1.3-8.5] and p=0.03, OR=2.2 [95% CI: 1.1-4.4], respectively). Relatedly, women with persistent other high-risk HPV types were more likely to have a lower CD4 count (p=0.01, OR=0.998 [95% CI: 0.99-1.0]).

Multivariate analysis was not performed for HPV16 persistence due to the low number of cases. In multivariate logistic regression for other high-risk type persistence, only CD4 count remained a significant predictor of persistence (p=0.02). The odds of other high-

risk HPV persistence are 16% lower for every 100 unit increase in CD4 count, after adjustment for viral load suppression.

The baseline cervical cytology within our cohort was 82.9% normal, 2.4% atypical squamous cells of undetermined significance (ASCUS), 11.5% low-grade squamous intraepithelial lesions (LSIL), 0.4% atypical squamous cells – cannot exclude HSIL (ASC-H) and 2.8% high-grade squamous intraepithelial lesions (HSIL) (Table 4.5). Of the seven HSIL cases observed, there were two in which only HPV35 was detected, one in which only HPV52 was detected, and one in which only HPV33 was detected. In three of the cases, multiple HPV infections were detected with the types listed in Table 4.5.

**4.4 Discussion**

In this cohort of 252 WLWH, the oncogenic HPV types responsible for the highest frequencies of persistent infections in this cohort were HPV16, 45, and 52, which are all contained in the nonavalent vaccine. However, in a population of persons living with HIV we observed persistent infection with oncogenic HPV types not contained within any currently available vaccine. These types included HPV56, 35, 39, 51, and 59 which were persistent in 4.8%, 3.6%, 3.6%, 3.2%, and 3.2% of women in the study, respectively. While these HPV types contribute less to cervical cancer than the types contained within available vaccines, HIV infection may increase the pathogenicity of these less common types in WLWH due to known interactions between HPV and HIV at the cervix (54, 126).

The rates of cytological outcomes seen in this population are similar to those seen in other engaged, North American populations of WLWH around the time of combination antiretroviral therapy (cART) implementation (114). As the cases of HSIL cytology are the

closest surrogate for cervical precancer/cancer outcomes, the HPV types present in these cases were of particular interest.

Although based on a small number of HSIL cytology cases, in our cross-sectional analysis (n=7), only a maximum of 29% of our HSIL cases were associated with highly oncogenic HPV16. We found that the other high-risk types were the cause of at least 29% of HSIL in our WLWH. Due to their presence in multiple-type infections, other high-risk types may have been responsible for up to 57% of the HSIL cytology cases. The relatively high rates of other high-risk types associated with HSIL cytology in this cohort, combined with relatively low rates of HPV16 are in accordance with literature that has found higher rates of less common oncogenic HPV types in WLWH compared to their HIV-negative counterparts (124, 127). This also supports the hypothesis that HPV16 has a reduced competitive advantage in the context of HIV infection and associated immune suppression (124).

The association of persistent other high-risk HPV infection with lower CD4 counts, a measure of immune function, reflects the importance of the immune system in clearing HPV infections rapidly prior to the establishment of persistence. CD4 count and HIV viral load are intrinsically related which explains the association to HIV viral load suppression seen in the univariate analyses for both HPV16 and other high-risk HPV persistence. Beyond the surrogacy of these measures for immune function, there may be an important role of virus-virus interactions in persistent infection. This may be related to the ability of HIV to disrupt the epithelial tight junctions, improving the ability of HPV to infect the basal epithelial cells (54). Additionally, it has been shown that HIV tat protein enhances HPV transcription and the expression of HPV oncogenes (126). Findings from our study also previously

demonstrated that immunogenicity to the quadrivalent HPV vaccine was higher in the context of HIV viral load suppression (105).

The high rate of persistent infection with HPV31/33/45/52/58 (types contained within the nonavalent vaccine but not within the quadrivalent or bivalent formulations; 17.9%) supports the added value of the nonavalent vaccine for WLWH. Importantly, there was a similar percentage of persistent infections caused by high-risk oncogenic HPVs not contained within any vaccine (17.1%). Thus, regardless of HPV vaccination history, it is critically important for clinicians to continue to offer cervical cancer screening to patients living with HIV.

As recruitment for this study occurred in HIV care clinics, the study population was generally well engaged in care and may not be generalizable to a less engaged population. This analysis was also limited by the fact that it only assessed two time points for HPV infection and was cross-sectional in its ascertainment of risk of cervical dysplasia by cytological testing. Additionally, the number of cytological HSIL cases was low which prevented us from determining attributable risk for each HPV type.

Higher CD4 count was associated with lower rates of HPV persistence and presumably lower risk of dysplasia (128). WLWH who had not yet received HPV vaccine in our study showed a wide range of oncogenic HPV infection, demonstrating the need to continue diligent cervical cancer screening in WLWH regardless of vaccine history.

**Table 4.1** Study Population Characteristics

| Characteristic | N (%) or median (IQR) |
|---|---|
| Age | 39 (33-45) |
| Ethnicity | |
| Black | 111 (44%) |
| Indigenous | 32 (13%) |
| White | 91 (36%) |
| Other | 18 (7%) |
| Region of origin | |
| Africa | 85 (34%) |
| Canada | 123 (49%) |
| Other | 44 (17%) |
| Probable mode of HIV acquisition | |
| Blood products | 14 (6%) |
| Sexual contact | 187 (74%) |
| IDU | 40 (16%) |
| MTCT | 7 (3%) |
| Other | 25 (10%) |
| Women with suppressed HIV viral load at baseline | 180 (73%) |
| CD4 count at baseline (/$mm^3$) | 510 (388-698) |
| CD4 nadir (/$mm^3$) | 240 (111-340) |

**Table 4.2** Prevalent and Persistent Infection

| Oncogenic HPV Genotype | Prevalent Infection | Persistent Infection |
|---|---|---|
| HPV 16 | 26 (10.3%) | 19 (7.5%) |
| HPV 45 | 18 (7.1%) | 16 (6.3%) |
| HPV 52 | 23 (9.1%) | 16 (6.3%) |
| HPV 56 | 16 (6.3%) | 12 (4.8%) |
| HPV 58 | 16 (6.3%) | 10 (4.0%) |
| HPV 35 | 12 (4.8%) | 9 (3.6%) |
| HPV 39 | 12 (4.8%) | 9 (3.6%) |
| HPV 18 | 14 (5.6%) | 8 (3.2%) |
| HPV 51 | 17 (6.7%) | 8 (3.2%) |
| HPV 59 | 15 (6.0%) | 8 (3.2%) |
| HPV 31 | 9 (3.6%) | 7 (2.8%) |
| HPV 68 | 13 (5.2%) | 6 (2.4%) |
| HPV 33 | 7 (2.8%) | 4 (1.6%) |
| HPV 82 | 5 (2.0%) | 3 (1.2%) |
| Any oncogenic HPV | 114 (45.2%) | 84 (33.3%) |

**Table 4.3** Persistent Infection by HPV Category

| **HR-HPV Category** | **N (%)** |
|---|---|
| HPV 16 | 19 (7.5%) |
| HPV 18 | 8 (3.2%) |
| HPV 31/33/45/52/58<br>(additional nonavalent) | 45 (17.9%) |
| HPV 35/39/51/56/59/68/82<br>(other high-risk, not in vaccines) | 43 (17.1%) |

**Table 4.4** Analysis of Factors Relating to HPV Persistence

| | **Persistent** Median (IQR) or n (%) | **Non-Persistent** Median (IQR) or n (%) | **Univariate analysis** p-value OR (95% CI) | **Multivariate analysis** p-value OR (95% CI) |
|---|---|---|---|---|
| **HPV16** | **N = 19** | **N = 233** | | |
| CD4 count | 403 (253-620) | 520 (450-620) | p=0.06<br>1 (0.99-1) | |
| CD4 nadir | 133 (88-251) | 240 (116-342) | p=0.18<br>1 (0.99-1) | |
| HIV viral load suppression | | | **p=0.02** | |
| Yes | 9 (47%) | 172 (74%) | 1.0 | |
| No | 10 (53%) | 59 (25%) | 3.2 (1.3-8.5) | |
| Unknown | 0 | 2 (1%) | n/a | |
| Age | 37 (29-42) | 39 (33-46) | **p=0.04**<br>0.95 (0.89-0.99) | |
| Ethnicity | | | p=0.21 | |
| Black | 6 (32%) | 105 (45%) | 0.3 (0.1-1.1) | |
| Indigenous | 5 (26%) | 27 (12%) | 1.0 | |
| Other | 8 (42%) | 101 (43%) | 0.4 (0.1-1.5) | |
| Region of origin | | | p=0.4 | |
| Africa | 4 (21%) | 81 (35%) | 1.0 | |
| Canada | 12 (63%) | 111 (48%) | 2.2 (0.7-8.1) | |
| Other | 3 (16%) | 41 (18%) | 1.5 (0.3-7.0) | |
| Total lifetime sexual partners | | | p=0.74 | |
| <5 | 6 (32%) | 75 (32%) | 1.0 | |
| 5-25 | 9 (47%) | 92 (39%) | 1.2 (0.4-3.8) | |
| 26-99 | 1 (5%) | 18 (8%) | 0.7 (0.04-4.4) | |
| >99 | 2 (11%) | 10 (4%) | 2.5 (0.3-12.7) | |
| Hepatitis C co-infection | | | p=0.47 | |
| Yes | 2 (11%) | 36 (15%) | 1.0 | |
| No | 17 (89%) | 181 (78%) | 1.7 (0.5-11.0) | |
| Unknown | 0 | 16 (7%) | n/a | |
| | | | | |
| **Additional Nonavalent HPV** | **N=45** | **N=207** | | |
| CD4 count | 521 (371-674) | 513 (380-673) | p=0.68<br>1.0 (0.99-1.0) | |
| CD4 nadir | 220 (110-328) | 240 (116-342) | p=0.90<br>1.0 (0.99-1.0) | |
| HIV viral load suppression | | | p=0.35 | |
| Yes | 30 (67%) | 151 (73%) | 1.0 | |
| No | 15 (33%) | 54 (26%) | 1.4 (0.7-2.8) | |
| Unknown | 0 | 2 (1%) | n/a | |
| Age | 39 (32-46) | 39 (34-45) | p=0.37<br>0.98 (0.9-1.0) | |
| Ethnicity | | | p=0.31 | |
| Black | 24 (53%) | 87 (42%) | 1.2 (0.5-3.5) | |
| Indigenous | 6 (13%) | 26 (13%) | 1.0 | |
| Other | 15 (33%) | 94 (45%) | 0.7 (0.3-2.1) | |

| | | | | |
|---|---|---|---|---|
| Region of origin | | | p=0.25 | |
| Africa | 19 (42%) | 66 (32%) | 1.0 | |
| Canada | 17 (38%) | 106 (51%) | 0.6 (0.3-1.2) | |
| Other | 9 (20%) | 35 (17%) | 0.9 (0.4-2.1) | |
| Total lifetime sexual partners | | | p=0.72 | |
| <5 | 18 (40%) | 63 (30%) | 1.0 | |
| 5-25 | 16 (36%) | 85 (41%) | 0.7 (0.3-1.4) | |
| 26-99 | 3 (7%) | 16 (8%) | 0.7 (0.1-2.3) | |
| >99 | 2 (4%) | 10 (5%) | 0.7 (0.1-3.0) | |
| Hepatitis C co-infection | | | p=0.97 | |
| Yes | 7 (16%) | 31 (15%) | 1.0 | |
| No | 37 (82%) | 161 (78%) | 1.0 (0.4-2.7) | |
| Unknown | 1 (2%) | 15 (7%) | n/a | |
| | | | | |
| **Other High-Risk HPV** | **N=43** | **N=209** | | |
| CD4 count | 440 (320-600) | 530 (392-696) | **p=0.01**<br>0.998 (0.99-1.0) | **p=0.02**<br>0.998 (0.99-1.0) |
| CD4 nadir | 185 (90-294) | 249 (120-350) | p=0.18<br>1.0 (1.0-1.0) | |
| HIV viral load suppression | | | **p=0.03** | p=0.09 |
| Yes | 25 (58%) | 156 (75%) | 1.0 | 1.0 |
| No | 18 (42%) | 51 (24%) | 2.2 (1.1-4.4) | 1.9 (0.9-3.8) |
| Unknown | 0 | 2 (1%) | n/a | n/a |
| Age | 38 (30-46) | 39 (34-45) | p=0.44<br>1.0 (1.0-1.0) | |
| Ethnicity | | | p=0.40 | |
| Black | 15 (35%) | 96 (46%) | 0.7 (0.3-2.0) | |
| Indigenous | 6 (14%) | 26 (12%) | 1.0 | |
| Other | 22 (51%) | 87 (42%) | 1.1 (0.4-3.2) | |
| Region of origin | | | p=0.58 | |
| Africa | 12 (28%) | 73 (35%) | 1.0 | |
| Canada | 24 (56%) | 99 (47%) | 1.5 (0.7-3.2) | |
| Other | 7 (16%) | 37 (18%) | 1.2 (0.4-3.1) | |
| Total lifetime sexual partners | | | p=0.67 | |
| <5 | 15 (35%) | 66 (32%) | 1.0 | |
| 5-25 | 15 (35%) | 86 (41%) | 0.8 (0.4-1.7) | |
| 26-99 | 2 (5%) | 17 (8%) | 0.5 (0.1-2.1) | |
| >99 | 3 (7%) | 9 (4%) | 1.5 (0.3-5.6) | |
| Hepatitis C co-infection | | | p=0.23 | |
| Yes | 4 (9%) | 34 (16%) | 1.0 | |
| No | 36 (84%) | 162 (78%) | 1.9 (0.7-6.6) | |
| Unknown | 3 (7%) | 13 (6%) | n/a | |

**Table 4.5** Attributable HPV in Baseline HSILs

| Oncogenic HPV Present | # HSIL Cases |
|---|---|
| 35 | 2 |
| 33 | 1 |
| 52 | 1 |
| Multiple infection<br>Case 1=16, 35, 56<br>Case 2=16, 58<br>Case 3=52, 56 | 3 |

# CHAPTER 5: IMMUNOLOGICAL IMPACT OF QUADRIVALENT HPV VACCINE DOSE SPACING IN WOMEN LIVING WITH HIV

## 5.1 Introduction

Human papillomavirus vaccines are safe, immunogenic, and effective (129). Initial dose schedules for the quadrivalent, bivalent, and nonavalent formulations recommended three doses administered at months zero, one or two, and six (130). However, more recent data have shown that young individuals who receive only two doses, and perhaps one dose, develop non-inferior peak antibody titers and may be adequately protected against persistent HPV infection and HPV-associated disease (83, 84). Additionally, studies have shown that expanding the time frame of the three-dose immunization schedule or delaying the second or third dose results in non-inferior immune responses in immunocompetent populations (131-134). Reduced-dose schedules can greatly expand the financial feasibility of HPV vaccination globally and will help reach the global target of cervical cancer elimination (106).

Given the global goal of cervical cancer elimination, consideration of all female populations is critical. A group of particular importance in achieving this goal is women living with HIV (WLWH). WLWH have higher rates of HPV infection and persistence, as well as an increased rate of HPV-associated disease (53, 65, 135). In addition, countries with high rates of HIV are also frequently the same countries with low rates of cervical cancer screening, and therefore are in the greatest need of simple, affordable vaccine schedules. The quadrivalent HPV (qHPV) vaccine has been shown to be safe, immunogenic, and efficacious in WLWH to date, with the greatest immune response seen in WLWH with suppressed HIV viral loads (105, 118). However, to our knowledge, no study to date has evaluated the effect

of qHPV vaccine dose spacing in this important population. In this study, we assess the effect of differential dose spacing of the qHPV vaccine on qHPV immunogenicity in WLWH.

### 5.2 Methods

Individuals aged nine and greater with a uterine cervix were recruited from 14 clinics serving WLWH across Canada between 2008-2012. The primary objective was to assess immunogenicity of the qHPV vaccine. The study population and methods of enrolment have been previously described (105). Participants provided informed consent and were scheduled to receive three doses of qHPV vaccine intramuscularly at month 0/2/6. Serology was performed at month 0/2/7/12/18/24 by cLIA assay at Merck Research Laboratories.

The objective of this analysis was to determine if the spacing of qHPV vaccine doses had an impact on the peak antibody titer achieved in WLWH. Peak antibody titer was defined as the highest anti-HPV antibody titer achieved in a participant, for each HPV type, post-vaccination. Peak titer was chosen as an endpoint as it is a direct measure of maximal vaccine response and is associated with protection against disease endpoints (136). Peak antibody titer was natural log transformed prior to analysis. Participants were divided into six groups based on vaccine spacing: one dose, two doses, three doses within seven months, three doses within seven months to one year, three doses within one to two years, and three doses over more than two years. We used Chi squared tests for trend for categorical variables and Spearman's rank correlation for continuous variables to assess differences in participant characteristics between vaccine spacing categories. Univariable and multivariable linear regression was performed for each qHPV type to examine if the spacing of the vaccine doses impacted the peak log titer achieved for each HPV type. Linear regression models included

potential confounders: age at first dose, time to blood draw following last dose, CD4 count and HIV viral load at first vaccine dose, CD4 nadir, and naivety to the HPV type. We also ran a sensitivity analysis excluding participants aged <14 years.

### 5.3 Results

The participants who received one dose (n=6), two doses (n=4), and three doses over greater than two years (n=5) were removed from analysis due to low numbers. Median peak log titers for one-dose recipients were 5.3, 5.3, 5.9, and 3.9 for HPV6, 11, 16, and 18, respectively. Median peak log titers for two-dose recipients were 6.9, 5.9, 6.9, and 4.9 for HPV6, 11, 16, and 18, respectively. Median peak log titers for recipients of three doses over greater than two years were 6.8, 6.9, 7.4, and 5.4 for HPV6, 11, 16, and 18, respectively. The number of participants in each group was small and participants differed between groups in their naivety to the HPV types, the time to serology following last vaccine dose, and other factors that may influence the peak log titer achieved, which cannot be properly assessed with our sample size. Participants who did not have serology performed after their last dose of vaccine were also removed due to the inability to assess the endpoint of interest (n=31).

After these exclusions, 307 participants were eligible for analysis (Table 5.1). The median age was 36 years (IQR: 26-44). Participants were predominantly of Black (47%) and White (32%) ethnicity. Four percent of participants had CD4 counts below 200 cells/$mm^3$ at baseline (time of first vaccination) and 50% had CD4 counts above 500 cells/$mm^3$. Sixty-six percent of participants had HIV plasma viral loads >50 copies/ml at baseline. Sixty-five percent of participants were naïve to HPV16 at baseline and median peak HPV16 log titer among all participants was 7.7 (IQR: 6.9-8.4).

Two hundred and twenty-nine participants received three doses of vaccine within 7 months, 56 received three doses within seven months to one year, and 22 received three doses within one to two years. Variables of interest were not significantly different between spacing categories (Table 5.1) with the exception of baseline CD4 count, naivety to HPV11, and time to blood draw post last vaccine dose. CD4 counts and rates of naivety to HPV11 decreased in the groups as the vaccine spacing increased. There was a similar but not significant trend for HPV16 naivety but no clear trend in HPV6 and 18. The time to blood draw post last dose was significantly greater as the vaccine spacing increased.

Univariable linear regression results suggested that HIV viral load suppression and time to blood draw were significantly associated with peak antibody titer for all qHPV types (Table 5.2). Peak antibody titers were 41-64% lower on average, depending on qHPV type, in participants with unsuppressed HIV viral loads, compared to those with suppressed HIV viral loads. As time between last vaccine dose and blood draw for serology increased, peak antibody response decreased, as expected based on immune titer dynamics. For all qHPV types aside from 16, previous exposure to the relevant HPV type was associated with 60-98% higher peak antibody titers, depending on qHPV type. The same trend was present for HPV16 although it did not reach significance. Higher baseline CD4 count was significantly associated with higher peak antibody titers for HPV16 and 18. Additionally, older age was associated with lower peak antibody titers for HPV16 only. Wider vaccine dose spacing was associated with lower peak antibody titers for HPV18; participants who received all doses within 7 months to 1 year had non-significantly higher peak antibody titers but participants who received all doses within 1 to 2 years had significantly lower peak antibody titers.

Multivariable linear regression yielded a significant relationship for all qHPV types between peak antibody titer and HIV viral load, naivety to the relevant HPV type, and time to blood draw (Table 5.2). Of note, there were no significant associations between peak antibody titers and spacing of vaccine doses in multivariable analysis. Participants with unsuppressed HIV viral loads had peak antibody titers that were 39-64% lower on average, depending on qHPV type. Participants who were previously exposed to the relevant HPV type had peak antibody titers that were 44-77% higher on average, depending on qHPV type, compared to naïve participants. As time between last vaccine dose and blood draw for serology increased, peak antibody response decreased. There was also a significant relationship between peak antibody titer and age, but only for HPV16 and 18, in which lower age was associated with higher peak antibody response.

A sensitivity analysis excluded 33 girls aged 13 and younger to assess the impact of age in the non-pediatric subset of the cohort. Girls aged 13 and younger were not analyzed separately due to low numbers and lack of diversity of dosing schedules (31 girls had all doses within seven months and two girls had all doses within seven months to one year). After removing girls aged 13 and younger, the median age was 37 (IQR: 31-44, n=274). The proportion of all other characteristics described in Table 5.1 did not significantly change. Multivariable linear regression of this non-pediatric subset demonstrated a significant relationship between peak antibody titer and time to blood draw, naivety to the relevant HPV type, and HIV viral load for all four HPV types, as it had for the full population. Again, there was no significant association between peak antibody titer and spacing of vaccine doses. There remained a significant relationship between peak antibody titer and age, only for HPV18.

## 5.4 Discussion

Of most importance to this analysis, vaccine dose spacing did not appear to be associated with peak antibody response in multivariable analyses for all qHPV types. Although peak log titers do appear to decrease slightly in median value as doses become more widely spaced, particularly for HPV16 and 18 (Table 5.1), the difference is not significant and can be accounted for by other variables in the models. The variables that did reach significance in our models are much more important to achieving a high antibody level in WLWH.

Time to blood draw, naivety to the relevant HPV type, and HIV viral load were important predictors of peak antibody response in univariable, multivariable, and sensitivity analyses. The relationship between age and peak antibody response was less clearly defined, with age not being a significant predictor of antibody response to HPV6, 11, but being a significant predictor of antibody response to HPV16 and 18. A previous study in women without HIV has noted the same association between increasing age and decreasing peak HPV18 antibody titer (137). Additionally, this finding is supported by biological understandings of how the immune system changes with age, resulting in reduced immunogenicity to many vaccines (138). The relationship between time to blood draw following last vaccine dose and peak antibody titer was expected due to known antibody titer dynamics, i.e. a decline and plateau following the final vaccine dose (88), and therefore needed to be included in the models.

We were limited in our ability to assess the impact of reduced dose schedules in this cohort due to high vaccine schedule adherence leading to small numbers of participants in the

one and two-dose groups. Due to the fact that most participants received all doses within seven months, further studies are needed to confirm our finding that the spacing of three dose schedules, up to a range of two years, does not meaningfully impact peak antibody response. Additional studies are also needed to assess the immunogenicity of one and two-dose schedules in WLWH. An additional limitation of this analysis was the use of antibody titers as a surrogate outcome in place of infection and disease endpoints, but low rates of these endpoints precluded their use.

These findings are critical to vaccine programming and provision globally. Our analysis suggests that deviations in vaccine dose spacing up to two years do not meaningfully impact peak antibody titer; therefore, restarting the vaccine schedule or adding an additional dose in these situations is not needed. As previously described (105), achieving HIV viral load suppression should be a key goal not only for HIV disease control and transmission prevention but also to achieve optimal HPV vaccine immune response. Additionally, these findings pertain to WLWH, who accounted for approximately 17.4 million of the women aged 15 and older globally in 2014 (139). Reaching the global target of cervical cancer elimination will require specific intervention targeting this important subset of women who experience a much higher burden of HPV and cervical cancer.

### 5.4.1 Conclusion

Taking into account age, time to serology, CD4 cell count, CD4 nadir, HIV viral load, and HPV naivety, the spacing of the three qHPV vaccine doses did not significantly impact peak anti-HPV antibody titers.

**Table 5.1** Participant Characteristics by Vaccine Spacing Category

| **Characteristic** | **Within 7 months** (n=229) | **7 months- 1 year** (n=56) | **1 to 2 years** (n=22) | **Linear-by-linear or Spearman's rank coefficient test** (p value) |
|---|---|---|---|---|
| Baseline age (years)+ | 37 (23-44) | 35 (30-38) | 36 (30-40) | 0.47 |
| Baseline CD4 count (cells/$mm^3$) | | | | |
| <200 | 7 (3%) | 4 (7%) | 2 (9%) | 0.02 |
| 200-500 | 86 (38%) | 24 (43%) | 12 (55%) | |
| >500 | 122 (53%) | 25 (45%) | 8 (36%) | |
| Baseline CD4 nadir (cells/$mm^3$)+ | 240 (122-370) | 255 (160-356) | 200 (80-320) | 0.64 |
| Baseline HIV viral load suppressed (<50 copies/mL) | | | | |
| Yes | 158 (69%) | 33 (59%) | 13 (59%) | 0.09 |
| No | 58 (25%) | 18 (32%) | 9 (41%) | |
| Unknown | 13 (6%) | 5 (9%) | 0 | |
| Naïve to HPV6 | | | | |
| Yes | 149 (65%) | 27 (49%) | 14 (64%) | 0.71 |
| No | 80 (35%) | 29 (52%) | 8 (36%) | |
| Naïve to HPV11 | | | | |
| Yes | 195 (85%) | 39 (70%) | 13 (59%) | <0.01 |
| No | 34 (15%) | 17 (30%) | 9 (41%) | |
| Naïve to HPV16 | | | | |
| Yes | 155 (68%) | 35 (63%) | 11 (50%) | 0.09 |
| No | 74 (32%) | 21 (38%) | 11 (50%) | |
| Naïve to HPV18 | | | | |
| Yes | 197 (86%) | 40 (71%) | 19 (86%) | 0.43 |
| No | 32 (14%) | 16 (29%) | 3 (14%) | |
| Time to blood draw post-last dose (days)+ | 31 (28-39) | 43 (30-100) | 80.5 (41-177) | <0.001 |
| Peak HPV6 log titer+ | 6.4 (5.5-7.1) | 6.7 (5.6-7.3) | 6.1 (5.2-7.1) | 0.57 |
| Peak HPV11 log titer+ | 6.4 (5.8-7.1) | 6.5 (5.8-7.1) | 6.3 (5.5-6.6) | 0.57 |
| Peak HPV16 log titer+ | 7.8 (7.1-8.4) | 7.6 (6.7-8.4) | 7.2 (5.6-8.0) | 0.05 |
| Peak HPV18 log titer+ | 5.9 (4.8-6.8) | 5.8 (4.8-6.7) | 5.1 (4.2-5.8) | 0.14 |

+ median, interquartile range

**Table 5.2** Univariable and Multivariable Linear Regression Results for Each HPV Type Assessing the Impact of Each Variable on Peak HPV Log Titer

| | **Univariable linear regression** | | | | **Multivariable linear regression** | | | |
|---|---|---|---|---|---|---|---|---|
| **HPV6** | | | | | | | | |
| **Variable** | **P value** | **β[a]** | **Exp β[b]** | **95% CI[c]** | **P value** | **β** | **Exp β** | **95% CI** |
| Baseline age (years, in increments of 10) | 0.07 | -0.10 | 0.91 | 0.82-1.01 | 0.11 | -0.12 | 0.88 | 0.75-1.03 |
| Baseline CD4 count (cells/mm$^3$) | 0.29 | | | | 0.77 | | | |
| <200 | | Ref | Ref | - | | Ref | Ref | - |
| 200-500 | | 0.34 | 1.41 | 0.70-2.85 | | 0.25 | 1.29 | 0.63-2.61 |
| >500 | | 0.49 | 1.63 | 0.81-3.28 | | 0.23 | 1.26 | 0.61-2.61 |
| Baseline CD4 nadir (cells/mm$^3$, in increments of 100) | 0.58 | 0.02 | 1.02 | 0.95-1.09 | 0.87 | -0.008 | 0.99 | 0.90-1.09 |
| Baseline HIV viral load suppressed (<50 copies/mL) | <0.001 | | | | 0.005 | | | |
| Yes | | Ref | Ref | - | | Ref | Ref | - |
| No | | -0.52 | 0.59 | 0.44-0.80 | | 0.49 | 0.61 | 0.43-0.87 |
| Naïve to HPV6 | 0.001 | | | | <0.001 | | | |
| Yes | | Ref | Ref | - | | Ref | Ref | - |
| No | | 0.48 | 1.61 | 1.20-2.16 | | 0.57 | 1.77 | 1.32-2.38 |
| Time to blood draw post-last dose (days) | <0.001 | -0.004 | 0.99 | 0.99-1.00 | <0.001 | -0.004 | 0.99 | 0.99-1.00 |
| Dose spacing category | 0.40 | | | | 0.17 | | | |
| Within 7 months | | Ref | Ref | - | | Ref | Ref | - |
| 7 months-1 year | | 0.16 | 1.17 | 0.82-1.67 | | 0.35 | 1.42 | 0.97-2.09 |
| 1-2 years | | -0.25 | 0.78 | 0.46-1.33 | | 0.22 | 1.25 | 0.71-2.18 |
| **HPV11** | | | | | | | | |
| **Variable** | **P value** | **β** | **Exp β** | **95% CI** | **P value** | **β** | **Exp β** | **95% CI** |
| Age (years, in increments of 10) | 0.36 | -0.05 | 0.95 | 0.86-1.06 | 0.56 | -0.04 | 0.96 | 0.83-1.11 |
| CD4 count (cells/mm$^3$) | 0.17 | | | | 0.71 | | | |
| <200 | | Ref | Ref | - | | Ref | Ref | - |
| 200-500 | | 0.42 | 1.52 | 0.77-3.02 | | 0.20 | 1.22 | 0.63-2.35 |
| >500 | | 0.58 | 1.79 | 0.91-3.52 | | 0.10 | 1.10 | 0.56-2.18 |
| CD4 nadir (cells/mm$^3$, in increments of 100) | 1.00 | 9.9e-6 | 1.00 | 0.94-1.07 | 0.86 | 0.007 | 1.01 | 0.92-1.10 |
| HIV viral load suppressed (<50 copies/mL) | <0.001 | | | | <0.001 | | | |
| Yes | | Ref | Ref | - | | Ref | Ref | - |
| No | | -0.75 | 0.47 | 0.35-0.62 | | 0.81 | 0.44 | 0.32-0.61 |
| Naïve to HPV11 | 0.007 | | | | <0.001 | | | |
| Yes | | Ref | Ref | - | | Ref | Ref | - |
| No | | 0.47 | 1.60 | 1.14-2.25 | | 0.56 | 1.74 | 1.25-2.43 |
| Time to blood draw | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| post-last dose (days) | <0.001 | -0.005 | 0.99 | 0.99-1.00 | <0.001 | -0.004 | 0.99 | 0.99-1.00 |
| Dose spacing category | | | | | | | | |
| Within 7 months | 0.52 | Ref | Ref | - | 0.81 | Ref | Ref | - |
| 7 months-1 year | | -0.05 | 0.95 | 0.67-1.35 | | 0.08 | 1.08 | 0.75-1.55 |
| 1-2 years | | -0.30 | 0.74 | 0.44-1.25 | | 0.15 | 1.16 | 0.69-1.97 |
| **HPV16** | | | | | | | | |
| **Variable** | **P value** | **β** | **Exp β** | **95% CI** | **P value** | **β** | **Exp β** | **95% CI** |
| Age (years, in increments of 10) | 0.009 | -0.16 | 0.86 | 0.76-0.96 | <0.001 | -0.24 | 0.78 | 0.69-0.88 |
| CD4 count (cells/mm$^3$) | | | | | | | | |
| <200 | 0.05 | Ref | Ref | - | 0.69 | Ref | Ref | - |
| 200-500 | | 0.14 | 1.15 | 0.53-2.50 | | -0.1 | 0.91 | 0.45-1.85 |
| >500 | | 0.51 | 1.67 | 0.77-3.60 | | 0.04 | 1.04 | 0.50-2.15 |
| CD4 nadir (cells/mm$^3$, in increments of 100) | 0.56 | 0.02 | 1.02 | 0.95-1.10 | 0.23 | -0.05 | 0.95 | 0.88-1.03 |
| HIV viral load suppressed (<50 copies/mL) | | | | | | | | |
| Yes | <0.001 | Ref | Ref | - | <0.001 | Ref | Ref | - |
| No | | -0.72 | 0.49 | 0.35-0.67 | | -0.66 | 0.52 | 0.37-0.71 |
| Naïve to HPV16 | | | | | | | | |
| Yes | | | | | | | | |
| No | 0.17 | Ref | Ref | - | 0.02 | Ref | Ref | - |
| | | 0.22 | 1.25 | 0.91-1.72 | | 0.36 | 1.44 | 1.07-1.94 |
| Time to blood draw post-last dose (days) | <0.001 | -0.007 | 0.99 | 0.99-0.99 | <0.001 | -0.01 | 0.99 | 0.99-0.99 |
| Dose spacing category | | | | | | | | |
| Within 7 months | 0.08 | Ref | Ref | - | 0.95 | Ref | Ref | - |
| 7 months-1 year | | -0.23 | 0.80 | 0.54-1.18 | | 0.05 | 1.06 | 0.73-1.53 |
| 1-2 years | | -0.62 | 0.54 | 0.30-0.98 | | 0.06 | 1.06 | 0.60-1.87 |
| **HPV18** | | | | | | | | |
| **Variable** | **P value** | **β** | **Exp β** | **95% CI** | **P value** | **β** | **Exp β** | **95% CI** |
| Age (years, in increments of 10) | 0.07 | -0.12 | 0.89 | 0.78-1.01 | 0.02 | -0.21 | 0.81 | 0.68-0.97 |
| CD4 count (cells/mm$^3$) | | | | | | | | |
| <200 | 0.03 | Ref | Ref | - | 0.92 | Ref | Ref | - |
| 200-500 | | 0.17 | 1.18 | 0.50-2.80 | | -0.002 | 1.00 | 0.46-2.17 |
| >500 | | 0.61 | 1.84 | 0.79-4.32 | | 0.07 | 1.07 | 0.48-2.39 |
| CD4 nadir (cells/mm$^3$, in increments of 100) | 0.99 | $6.9x10^{-4}$ | 1.00 | 0.92-1.08 | 0.75 | -0.02 | 0.98 | 0.89-1.09 |
| HIV viral load suppressed (<50 copies/mL) | | | | | | | | |
| Yes | <0.001 | Ref | Ref | - | <0.001 | Ref | Ref | - |
| No | | -1.02 | 0.36 | 0.25-0.52 | | 1.03 | 0.36 | 0.25-0.52 |
| Naïve to HPV18 | | | | | | | | |
| Yes | 0.003 | Ref | Ref | - | 0.007 | Ref | Ref | - |
| No | | 0.68 | 1.98 | 1.27-3.09 | | 0.55 | 1.74 | 1.15-2.63 |
| Time to blood draw | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| post-last dose (days) | <0.001 | -0.007 | 0.99 | 0.99-0.99 | <0.001 | -0.006 | 0.99 | 0.99-1.00 |
| Dose spacing category | | | | | | | | |
| Within 7 months | 0.02 | Ref | Ref | - | 0.51 | Ref | Ref | - |
| 7 months-1 year | | 0.02 | 1.02 | 0.66-1.59 | | 0.15 | 1.16 | 0.76-1.77 |
| 1-2 years | | -0.91 | 0.40 | 0.21-0.77 | | -0.24 | 0.79 | 0.43-1.46 |

[a] Coefficients from the linear regressions in natural log units of peak HPV antibody titer.
[b] Exponentiated coefficients ($e^{coefficient}$) from the linear regression models. This gives the results in terms of a ratio of geometric mean peak antibody titers on the original scale [log(A) – log(B) = log(A/B)], and can be interpreted as percent difference (e.g. 0.80 would indicate that the peak antibody titers were 20% lower on average in the category of interest compared to the reference category, while a value of 2 would indicate that the peak antibody titer was 200% (2 times) higher in the category of interest compared to the reference category. A value of 1 indicates no difference between the groups in antibody titers).
[c] 95% CI of the exponentiated coefficients.

# CHAPTER 6: THE EFFICACY OF THE QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE IN GIRLS AND WOMEN LIVING WITH HIV

## 6.1 Introduction

Cervical cancer is a major health burden for women, resulting in over 250 000 deaths globally each year (140). While low- and middle-income countries bear the greatest burden of disease, with an age-standardized cervical cancer mortality rate of 8.3 per 100 000 population, many women in high-income countries (HIC) also continue to be affected, with a mortality rate of 3.3 per 100 000 (141). Human papillomavirus (HPV) has been well established as the primary causal agent of cervical cancer, making this cancer a vaccine-preventable disease (13, 142).

HPV is also the causal agent of genital warts, a widespread problem with a global annual incidence of 195 per 100 000 population (24). Although genital warts are not life threatening, they are one of the most frequent sexually transmitted infections, resulting in negative quality of life consequences (29, 30).

Women living with HIV (WLWH) are disproportionately affected by HPV infection and cervical cancer, with HIV infection being an independent risk factor for cervical cancer (59, 143). WLWH have a 47-53% prevalence of HPV infection, which is approximately double the prevalence among women without HIV (22-29%) (53, 114). Compared to invasive cervical cancers (ICCs) in women without HIV, ICCs in WLWH have a higher prevalence of oncogenic HPV types other than HPV16 and 18, which appears to be due to higher rates of multiple HPV infection among WLWH (144). Despite widespread screening programs in HIC, WLWH continue to experience higher and more rapid rates of progression

to high-grade cervical dysplasia and ICC than women without HIV. Among North American women, WLWH have an ICC incidence rate of 26 per 100 000 person-years, compared to 6 per 100 000 person-years in women without HIV (122). WLWH are also more likely to experience larger and more recurrent warts; genital wart incidence was reported as 5.0 per 100 person-years in WLWH compared to 1.3 per 100 person-years in women without HIV (145).

HPV vaccines exist in bivalent, quadrivalent, and nonavalent formulations. These vaccines have shown a high degree of safety, immunogenicity, and efficacy in HIV-negative populations (88, 90-93, 137, 146-152). HPV vaccine safety and immunogenicity results within populations with HIV appear promising (101-103), with data from our cohort showing 94-99% seroconversion and improved peak geometric mean titres (GMTs) in participants with HIV virologic suppression compared to those not suppressed (105). There has been a prior publication of HPV vaccine efficacy in persons living with HIV (153), but none to date of WLWH and none with cervical disease endpoints. This analysis assesses the 2-year efficacy of the quadrivalent HPV (qHPV) vaccine in a cohort of WLWH. As no immune correlate of protection for HPV has been established, efficacy findings are critical to better understand how the HPV vaccine performs in individuals with HIV. HPV vaccines are currently offered in HIV-endemic countries without HIV-specific efficacy data to support schedule recommendations for individuals with HIV. As the first report of infection and histological outcomes in WLWH post HPV vaccination, these findings will inform vaccine rollout for this population globally.

## 6.2 Methods

### 6.2.1 Study population

Girls and WLWH were recruited from 14 HIV clinics across Canada between 2008-2012, as described in a previous publication (105). Eligibility included: aged 9 years or older, not pregnant, willing to avoid pregnancy during the vaccination series, and had to have a cervix. Recruited individuals were ineligible if they had received any HPV vaccine, had an allergy to vaccine components, were currently enrolled in a trial of an investigational drug or vaccine, or if a site investigator deemed their health to be exclusionary. Participants, or guardians, provided voluntary informed consent to participate.

### 6.2.2 Study Design

Participants were asked to attend 8 study visits: one screening visit (-3 months) and 7 study visits (month 0/2/6/7/12/18/24), and were to receive three doses of qHPV vaccine intramuscularly at month 0/2/6. Pelvic examination was performed on participants who were post-menarchal and sexually active. Cervical cytology and cervico-vaginal HPV DNA samples were collected at screening and at month 0/6/12/18/24. Cervical cytology samples were collected using ThinPrep® Pap Test and were classified by Bethesda Criteria at the British Columbia Cancer Agency Cervical Cancer Screening Laboratory. For HPV DNA detection, cervico-vaginal samples collected in PreservCyt® were processed and typed for 36 HPV genotypes by Linear array assay (Roche Molecular Systems) (125). Participants were referred for colposcopies as per regional recommendations. Histological diagnoses were collected from pathology reports of individuals who underwent colposcopy with cervical biopsy and/or endocervical curettage.

### 6.2.3 Statistical Methods

In this efficacy analysis, newly acquired persistent HPV infection was defined as the detection of the same qHPV type (i.e., HPV type protected against by the qHPV vaccine; HPV6/11/16/18) in samples collected at two or more consecutive visits (>6 months apart) or detection of qHPV at the last available visit (136).

The second endpoint was incident cervical intraepithelial neoplasia of grade 2 and higher (CIN2+). Participants considered for this endpoint had to have normal baseline cytology. A third endpoint was incident genital warts and participants had to have no genital warts present at baseline to be considered for this endpoint. Duration of follow-up for the endpoints varies due to the differing inclusion criteria.

Analyses were undertaken in three sub-populations. The per-protocol efficacy (PPE) population included those who received all three doses of vaccine within 1 year and who had at least one follow-up visit including pelvic examination after month 7 post initial vaccination. Participants had to be naïve to the relevant qHPV type at baseline by competitive Luminex immunoassay and Linear array assay (i.e., antibody and DNA negative). Case counting for this population began at month 7. A naïve to relevant type (NRT) population and an intention-to-treat (ITT) population were also considered. Participants in the NRT population received at least one dose of vaccine, attended at least one follow-up visit with pelvic examination after day 1, and were naïve to the relevant qHPV type at baseline. Participants included in the ITT group received at least one dose of vaccine and attended at least one follow-up visit with pelvic examination after day 1. Case counting

began on day 1 for participants in the NRT and ITT analyses. These sub-population definitions were consistent with prior definitions used in the HPV vaccine literature (91).

Due to the known safety and efficacy of the qHPV vaccine in the pre-licensure trials, it was unethical to perform a placebo-controlled study. However, comparisons were drawn between our cohort and a cohort of women without HIV to provide context for our results. The most suitable group for comparison was that of Muñoz et al., 2009 which had a similar median follow-up time of 2.2 years and age range of 24-45 years (median=35, n=1911) (91). In order to improve similarity of our cohort to this comparator group, PPE, NRT, and ITT sub-populations for comparison were created in which participants from our cohort were excluded if they had a history of genital warts, history of cervical disease, or past cervical surgical procedure as these women would have been ineligible for the Muñoz et al. study. The comparator group utilized a composite endpoint of persistent qHPV, external genital disease, or cervical disease associated with qHPV types. Results for the same composite endpoint were procured within our vaccinated WLWH to assess differences. The definitions of these endpoints were consistent between studies.

Comparison was also made to unvaccinated WLWH from a previous study, the Canadian Women's HIV Study (CWHS) (114). CWHS followed 750 WLWH in the pre-HPV vaccine era (1993-2002) and had the same median follow-up time of 2 years, a similar median age of 33 (interquartile range [IQR]: 28-38), a similar ethnic makeup, and participants received their care at many of the same clinics across Canada (114).

### 6.3 Results

420 girls and women were enrolled in this national observational study. Of those enrolled, 279 women met inclusion criteria for at least one sub-population of this 2-year efficacy analysis; reasons for non-inclusion are described in Figure 6.1. Baseline characteristics of eligible participants are shown in Table 6.1. The median age was 39 years (IQR: 34-45, range: 13-66). Participants were ethnically mixed but predominantly Black (41.9%) and White (36.2%). The region of origin was predominantly Canada (50.5%), followed by Africa (33.3%). The median CD4 count at first vaccination was 500 cells/$mm^3$ (IQR: 380-682) and 69% of participants had HIV plasma viral loads <50 copies/mL. 266 (95.3%) received all three doses of vaccine, 7 (2.5%) received 2 doses, and 6 (2.2%) received 1 dose. At baseline, the most frequently detected HPV types were HPV16 (10.3%), HPV52 (9.1%), and HPV45 (7.1%). Prevalent HPV18 infection was only seen in 5.6% of participants. The vaccine was found to be safe and highly immunogenic within this population, as previously described (105).

Among women in the ITT group (Table 6.2), 11 cases of newly acquired persistent qHPV were observed in 477.7 person-years of follow-up (median follow-up 2 years, IQR: 1.6-2.1). The incidence rate of this endpoint was 2.3 per 100 person-years (95% confidence interval [CI]: 1.1-4.1). Six of the persistent qHPV infections were HPV18, three were HPV6, one was HPV11, and one was HPV16. The incidence rate of genital warts was 2.3 per 100 person-years (95% CI: 1.2-4.1). No cases of qHPV-associated CIN2+ were seen in women with normal baseline cytology.

Within the NRT population, the incidence rate of newly acquired persistent qHPV was 1.1 per 100 person-years (95% CI: 0.3-2.5) and the incidence rate of genital warts was

2.1 per 100 person-years (95% CI: 1.0-3.9). All cases of persistent qHPV were due to HPV18. No cases of CIN2+ were observed.

Among 212 women eligible for the PPE population, the incidence rate of newly acquired persistent qHPV was 1.0 per 100 person-years (95% CI: 0.3-2.6). All four cases of persistent qHPV were due to HPV18. No cases of qHPV-associated CIN2+ developed among women with normal baseline cytology. There were, however, 2 cases of cytological HSIL, 1 atypical glandular cells (AGC), and 1 atypical squamous cells – cannot exclude HSIL (ASC-H) in women with normal baseline cytology. None of these abnormal cytology results were qHPV-associated. Within the PPE population, the incidence rate of genital warts was 1.0 per 100 person-years (95% CI: 0.3-2.5). Of the four genital wart cases, three were HPV6 DNA-positive at baseline and one had a history of warts and was HPV6 DNA-positive at the time of wart detection. As such, these newly clinically recognized warts were likely due to pre-existing infection.

Although there were too few events of vaccine failure within the PPE group to assess predictors in a statistically robust manner, some trends were observed (Table 6.3). The eight cases had a median baseline CD4 count of 333 cells/mm$^3$ (IQR: 298-435), which was lower than the median of 513 cells/mm$^3$ (IQR: 390-700) among women who had not experienced vaccine failure. Similarly, the median CD4 nadir of those who experienced vaccine failure (37 cells/mm$^3$, IQR: 32-283) was lower than the median CD4 nadir of those who did not (240 cells/mm$^3$, IQR: 133-339). Among those who experienced breakthrough persistent qHPV, all of which were HPV18, the median log peak HPV18 geometric mean titre (GMT) was 5.95 (IQR: 4.3-6.3), which was similar to the median of 5.87 (IQR: 4.8-6.7) for those who did not experience breakthrough.

It was notable that all four cases of breakthrough persistent qHPV in the PPE group were HPV18. In the NRT group, the same four cases of HPV18 were seen as well as one additional case of HPV18. As this is a statistically unlikely situation given the higher prevalence of HPV16 and 6 in the general population, this finding was further explored. This finding was not due to laboratory contamination as the samples were collected over the span of one year, did not undergo HPV DNA testing concurrently, and all negative controls during this year tested negative. Screening and baseline samples from these participants were re-tested with an HPV18-specific real time PCR assay to determine if these individuals were incorrectly classified as naïve to HPV18 at study initiation (154). The real time assay revealed that the one individual who was only in the NRT group was infected with HPV18 at screening and thereby did not represent vaccine failure. The sample contained a very low HPV18 copy number, which explains why it was previously negative via the less sensitive Linear array assay. All PPE cases remained classified as naïve to HPV18 at baseline with the real time PCR assay (data not shown). Of the four HPV18 cases, one was a persistent infection present at two consecutive study visits. In the remaining samples, HPV18 was only present in the last available sample.

Comparison to the Muñoz et al. cohort of women without HIV (91) showed that the rates of the composite endpoint (i.e., vaccine failure) were greater in our cohort of WLWH for the PPE group compared to the HIV-negative vaccinated PPE group (1.2 versus 0.1 per 100 person-years, rate ratio: 11.7 [95% CI: 2.6-52.1]), while not significantly greater when comparing the NRT or ITT groups (NRT rate ratio: 4.1, ITT rate ratio: 1.1) (Table 6.4). In fact, the composite endpoint rates within our groups of vaccinated WLWH were not different

from the HIV-negative placebo group rates (PPE rate ratio: 0.8; NRT rate ratio: 1.0; ITT rate ratio: 0.8).

We also compared the incidence rates of persistent qHPV, CIN2+, and genital warts to a cohort of unvaccinated WLWH from the CWHS conducted in the pre-HPV vaccine era (Table 6.5) (114). The rate of persistent qHPV is substantially lower among vaccinated WLWH compared to the historical unvaccinated group (2.3 versus 6.0 per 100 person-years). However, the rates of genital warts and CIN2+ do not differ as greatly (2.3 versus 2.9 per 100 person-years and 0 versus 1 per 100 person-years, respectively).

**6.4 Discussion**

The fact that our WLWH experienced rates of persistent qHPV and qHPV-related disease similar to those of an HIV-negative placebo group from the literature (91) suggests that WLWH may be at higher risk for vaccine failure than their HIV-negative counterparts. However, the rate of newly acquired persistent qHPV was significantly less than the rate seen in unvaccinated WLWH in the literature (114), which suggests that although protection is not as complete as that seen in women without HIV, an important benefit appears to be present.

Overall rates of vaccine failure were low within this cohort of WLWH. The fact that three out of four HPV18 breakthrough infections were cases in which the infection was present at the last available sample, and not persistent between two study visits, does not diminish the relevance of our findings because the definition of breakthrough persistent qHPV infection is consistent with other studies of HPV vaccine efficacy. The lack of any CIN2+ diagnoses thus far is encouraging but not a surprising finding at two years of follow-up as CIN2+ usually requires 7-10 years to develop in women without HIV (155); however,

this remains a promising finding, as median time to CIN2+ diagnosis has been reported to be as short as three years in women without HIV (156). Further follow-up is underway to assess longer-term efficacy of the vaccine within this cohort.

The disparities noted between the median baseline CD4 counts and CD4 nadirs of all PPE cases and non-cases suggest that present and historical immune dysfunction may contribute to breakthrough HPV infection and disease as a whole, not solely to HPV18 breakthrough. Higher case numbers are required to properly elucidate this relationship. The fact that a higher incidence of HPV-associated disease is seen in WLWH who have CD4 counts below 350 cells/μL supports the idea that impaired immune functionality caused by HIV may play a role in HPV persistence and disease (157) and that HPV-specific CD4 responses to the vaccine may be deficient in women with breakthrough infection and disease despite overall good immunogenicity in this cohort (105). Future studies assessing CD4-induced vaccine responses in WLWH would provide valuable insight. Importantly, comparisons between groups of women who experienced vaccine failure and those who did not may evolve as further vaccine failure may occur in time.

### 6.4.1 Strengths and Limitations

To our knowledge, this study is the first report of HPV vaccine efficacy against cervical infection and disease in WLWH, providing valuable insights towards prevention of HPV-associated disease in this population. Study limitations include moderate cohort size with relatively short follow-up time of two years, which affected our ability to produce highly precise confidence intervals. This cohort continues to be followed and future reporting on longer follow-up is forthcoming. Due to the ethical limitation of not using a placebo

group, our comparisons utilize comparable published data from an HIV-negative vaccinated cohort (91) and a historical group of WLWH (114). Collectively, we referred to the described endpoints as vaccine failures, however, it is possible that not all of these cases do represent true vaccine failure. Partner deposition of HPV could be responsible for the detection of some HPV cases. Recent literature suggests that only approximately 14% of HPV DNA detected in a cohort of Canadian women is due to recent vaginal sex (158).

### 6.4.2 Conclusions

Given the relatively low rate of vaccine failure within the first two years of follow-up, paired with a good safety and immunogenicity profile, the HPV vaccine should continue to be offered to a wide age range of WLWH. It is, however, important to recognize that WLWH appear to be at higher risk than women without HIV for acquiring persistent qHPV-related infection and disease despite vaccination against HPV. As a result, regular cervical screening remains important in vaccinated WLWH. Even though the protection may not be as complete, the rate of persistent qHPV is greatly diminished in vaccinated compared to unvaccinated WLWH. Longer-term follow-up will better inform vaccine schedule recommendations for this population.

**Table 6.1** Study Population Characteristics (n=279)

| Characteristic | N (%) or Median (IQR) |
|---|---|
| Age | 39 (34-45) |
| Ethnicity | |
| Asian | 15 (5.4%) |
| Black | 117 (41.9%) |
| Hispanic | 4 (1.4%) |
| Indigenous | 39 (14.0%) |
| White | 101 (36.2%) |
| Other | 3 (1.1%) |
| Region of origin | |
| Africa | 93 (33.3%) |
| Asia | 13 (4.7%) |
| Canada | 141 (50.5%) |
| Caribbean | 19 (6.8%) |
| Central America | 4 (1.4%) |
| Europe | 5 (1.8%) |
| South America | 4 (1.4%) |
| Total lifetime sexual partners | 6 (3-12) |
| Years since HIV diagnosis | 8 (4-12) |
| Baseline CD4 count (cells/$mm^3$) | 500 (380-682) |
| CD4 nadir (cells/$mm^3$) | 230 (118-339) |
| HIV viral load suppression (VL<50 copies/mL) | 192 (68.8%) |
| Unknown | 9 (3.2%) |
| ARV regimen status | |
| PI-based | 135 (48.4%) |
| NNRTI-based | 75 (26.9%) |
| Not yet started | 17 (6.1%) |
| Previously on ARVs | 10 (3.6%) |
| Other | 30 (10.8%) |
| Unknown | 11 (3.9%) |
| Baseline cytology | |
| Normal | 226 (81.0%) |
| ASCUS | 9 (3.2%) |
| LSIL | 25 (9.0%) |
| ASC-H | 1 (0.4%) |
| HSIL | 9 (3.2%) |
| No result | 9 (3.2%) |
| Number of vaccine doses | |
| 3 | 266 (95.3%) |
| 2 | 7 (2.5%) |
| 1 | 6 (2.2%) |

Abbreviations: IQR, interquartile range; ARV, antiretroviral; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells – cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion.

**Table 6.2** Incidence Rates of Study Endpoints within PPE, NRT, and ITT Populations

| | N | Cases | Person-years | Rate (95% CI) |
|---|---|---|---|---|
| **PPE population** | | | | |
| Breakthrough persistent qHPV | 212 | 4 | 396.5 | 1.0 (0.3-2.6) |
| Genital warts | 211 | 4 | 403.0 | 1.0 (0.3-2.5) |
| CIN2+ | 177 | 0 | 334.6 | 0 (0.0-1.1) |
| **NRT population** | | | | |
| Breakthrough persistent qHPV | 260 | 5 | 464.6 | 1.1 (0.3-2.5) |
| Genital warts | 258 | 10 | 467.1 | 2.1 (1.0-3.9) |
| CIN2+ | 210 | 0 | 375.9 | 0 (0.0-1.0) |
| **ITT population** | | | | |
| Breakthrough persistent qHPV | 268 | 11 | 477.7 | 2.3 (1.1-4.1) |
| Genital warts | 264 | 11 | 476.7 | 2.3 (1.2-4.1) |
| CIN2+ | 217 | 0 | 387.1 | 0 (0.0-0.9) |

Abbreviations: CI, confidence interval; qHPV, quadrivalent HPV (HPV6/11/16/18); CIN2+, cervical intraepithelial lesion of grade 2 or higher; PPE, per-protocol efficacy; NRT, naïve to relevant type; ITT, intention-to-treat.

**Table 6.3** PPE Vaccine Failure Listing

| Case Type | Baseline Age | Baseline CD4 Count (cells/mm$^3$) | CD4 Nadir (cells/mm$^3$) | Screening HIV Viral Load (copies/mL) | Baseline HIV Viral Load (copies/mL) | Time to Infection or Disease/ Duration of Follow-Up (years) | Log Peak HPV 18 GMT |
|---|---|---|---|---|---|---|---|
| qHPV | 20 | 430 | 400 | 425 | 20 027 | 2.0 | 5.37 |
| qHPV | 44 | 292 | 32 | <50 | <50 | 1.6 | 5.86 |
| qHPV | 49 | 320 | 33 | <50 | <50 | 1.8 | 6.95 |
| qHPV | 30 | 1570 | 767 | <50 | <50 | 2.0 | 6.03 |
| Wart | 47 | 130 | 40 | <50 | 96 952 | 1.6 | NA |
| Wart | 42 | 450 | 30 | <50 | NA | 0.6 | NA |
| Wart | 42 | 346 | 244 | <50 | <50 | 0.6 | NA |
| Wart | 27 | 300 | 30 | <50 | 71 | 1.5 | NA |
| Median of Cases | 42 | 333 | 37 | | | 1.6 | 5.95 |
| Median of Non-Cases | 39 | 513 | 240 | | | 2.0 | 5.87 |

Abbreviations: GMT, geometric mean titre; qHPV, quadrivalent HPV (HPV6/11/16/18).

**Table 6.4** Comparison of Composite Endpoint Rates in WLWH Versus Women Without HIV

| | Muñoz et al., 2009 Vaccinated HIV-negative | | | Muñoz et al., 2009 Placebo HIV-negative | | | Present study Vaccinated WLWH | | | Vaccinated WLWH vs Vaccinated HIV- | Vaccinated WLWH vs Placebo HIV- |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Cases of composite endpoint | Rate (per 100 person-years) | n | Cases of composite endpoint | Rate (per 100 person-years) | n | Cases of composite endpoint | Rate (per 100 person-years) | Rate ratio (95% CI) | Rate ratio (95% CI) |
| PPE | 1615 | 4 | 0.1 (0.02-0.03) | 1607 | 41 | 1.5 (1.1-2.0) | 137 | 3 | 1.2 (0.2-3.4) | 11.7 (2.6-52.1) | 0.8 (0.2-2.5) |
| NRT | 1841 | 20 | 0.5 (0.3-0.8) | 1833 | 77 | 2.0 (1.6-2.5) | 163 | 6 | 2.0 (0.7-4.5) | 4.1 (1.6-10.2) | 1.0 (0.4-2.3) |
| ITT | 1886 | 108 | 2.7 (2.2-3.3) | 1883 | 154 | 3.9 (3.3-4.6) | 167 | 9 | 3.0 (1.4-5.7) | 1.1 (0.6-2.2) | 0.8 (0.4-1.5) |

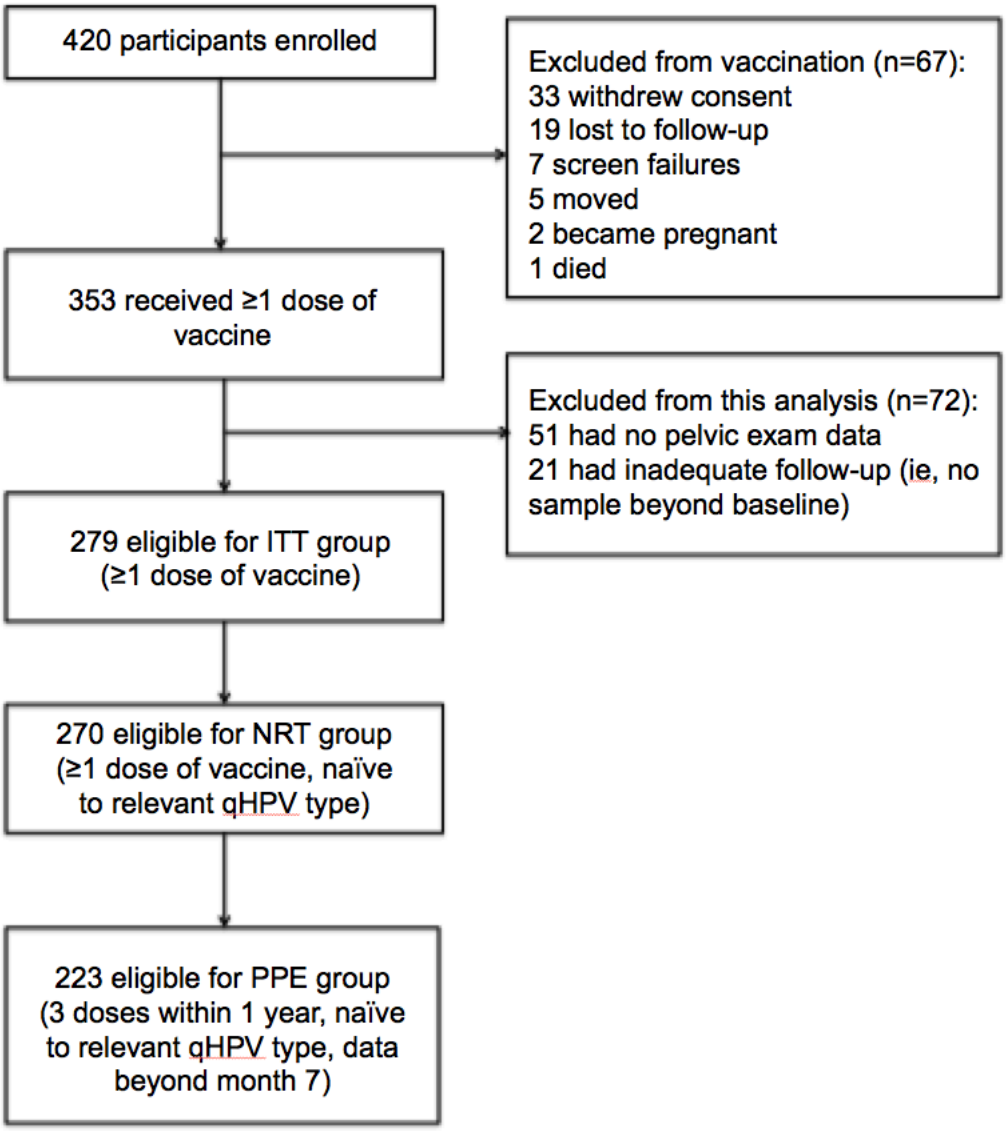
Abbreviations: PPE, per-protocol efficacy; NRT, naïve to relevant type; ITT, intention-to-treat; CI, confidence interval; WLWH, women living with HIV.

**Table 6.5** Comparison to Unvaccinated Historical WLWH

| | Unvaccinated Historical WLWH (Canadian Women's HIV Study) | Vaccinated WLWH (Present study) |
|---|---|---|
| **Endpoint** | **Rate per 100 person-years (95% CI)** | **Rate per 100 person-years (95% CI)** |
| Persistent qHPV | 6.0 (4.6-7.7) | 2.3 (1.1-4.1) |
| Genital warts | 2.9 (2.1-3.9) | 2.3 (1.2-4.1) |
| CIN2+ | 1.0 (0.5-1.9) | 0 (0.0-0.9) |

Abbreviations: WLWH, women living with HIV; CI, confidence interval; qHPV, quadrivalent HPV (HPV6/11/16/18); CIN2+, cervical intraepithelial lesion of grade 2 or higher.

**Figure 6.1** Flowchart of Study Participants




Abbreviations: ITT, intention-to-treat; NRT, naïve to relevant type; PPE, per-protocol efficacy; qHPV, quadrivalent HPV (HPV6/11/16/18).

# CHAPTER 7: PERSISTENCE OF NON-VACCINE ONCOGENIC HPV GENOTYPES IN QUADRIVALENT HPV-VACCINATED WOMEN LIVING WITH HIV

## 7.1 Introduction

Human papillomavirus (HPV) disproportionately affects women living with HIV (WLWH) resulting in a much larger burden of disease than that seen in the general population. The prevalence of HPV infection among WLWH is approximately 50%, which is twice the prevalence in women without HIV (114). The rate of persistent HPV infection among WLWH is approximately 20-24%, making WLWH 3-6 fold more likely to have a persistent HPV infection than women without HIV (135, 159). The disparity between rates of cervical cancer is equally wide; within a North American population of WLWH, the incidence rate of invasive cervical cancer was 16 per 100 000 person-years, compared to only 5 per 100 000 person-years in women without HIV (122).

In addition to higher rates of HPV-related infection and disease, WLWH also experience infection with a wider range of HPV types (64) which has important implications for vaccine and cervical screening programming. HPV16 is well known to be the most carcinogenic of HPV types. However, it is less affected by increased immunodeficiency than other oncogenic HPVs and is also seen in a reduced proportion among WLWH (124). Although HPV vaccines are now available and have promising safety and immunogenicity findings in WLWH to date (101-103, 105), it is critical to identify the residual burden of oncogenic HPV within WLWH in order to inform post-vaccination cervical screening needs for this population. In this study, we assessed rates of incident persistent infection with

oncogenic HPV types not contained in the quadrivalent HPV (qHPV) vaccine in our cohort of qHPV-vaccinated WLWH.

### 7.2 Methods

Girls and WLWH, aged nine and greater, were recruited from 14 clinics serving WLWH across Canada between 2008-2012 into a prospective longitudinal clinical study with long-term follow-up to six years. As there was no maximum age for this study, the vaccine was used beyond the age of FDA approval. All participants or guardians, as appropriate, provided informed consent to enroll in the study. The study population and methods of enrolment have previously been described (105). Participants were scheduled to receive three doses of qHPV vaccine intramuscularly at month 0/2/6. Serology was performed by cLIA assay at Merck Research Laboratories. Pelvic examination was performed on participants who were post-menarchal and sexually active. Cervical cytology and cervico-vaginal HPV DNA samples were collected at the screening visit and at months 0/6/12/18/24/36/48/60/72/84/96. The ThinPrep® Pap Test was utilized for collection of cervical cytology samples and results were classified by Bethesda Criteria centrally at the British Columbia Cancer Agency Cervical Cancer Screening Laboratory. Aliquots of the PreservCyt® from Pap tests were processed and typed for 36 HPV genotypes by Linear array assay (Roche Molecular Systems) (125).

The primary outcome of this analysis was rate of persistent HPV infection with non-quadrivalent vaccine HPV types (i.e., oncogenic types not including HPV6/11/16/18) within our cohort of qHPV-vaccinated WLWH. Persistent HPV infection was defined as the detection of the same incident HPV type in samples collected at two or more consecutive

study visits (>6 months apart) or detection of an HPV type at the last available visit. Although this definition of persistent infection is a common definition utilized within the HPV vaccine literature (160), it is known to overestimate the true number of persistent infections by including cases where HPV is present only in the last sample; however, it is accepted as it errs on the side of caution since some of these infections will persist. Due to this, a sensitivity analysis was also conducted where only the confirmed persistent cases (i.e., detection of the same HPV type at two or more consecutive study visits) were considered. The final sub-analysis presented herein determines the incidence of persistent infection with HPV types contained only in the nonavalent vaccine (HPV31/33/45/52/58) as compared to the incidence of persistent infection with oncogenic HPV types not contained within available vaccines (HPV35/39/51/56/59/68). To be eligible for this analysis, participants had to have received at least one dose of vaccine and had to have at least one HPV DNA result post-vaccination. For ascertainment of HPV cases, participants were required to be DNA negative to the relevant HPV type at the screening and baseline visits. The HPV types considered in this analysis were HPV31/33/35/39/45/51/52/56/58/59/68; these HPV types were selected for consideration due to their oncogenic potential (161).

### 7.3 Results

284 participants were eligible for analysis with 1205 person-years of follow-up and a median follow-up time of four years per person. Eligible population characteristics at baseline are described in Table 7.1. The median age was 38 years (IQR: 32-44). Participants were predominantly of Black (41%) and White (36%) ethnicity. The median CD4 count at first vaccination was 499 cells/$mm^3$ (IQR: 375-680) and 71% of participants had HIV plasma

viral loads <50 copies/ml. 267 participants (94%) received all three doses of vaccine. The vaccine was safe and highly immunogenic within this population, as previously described (105).

The incidence rates of persistent HPV types are shown in Figure 7.1. The most frequently documented persistent infections were infections with HPV51 (incidence rate [IR]: 1.4 per 100 person-years [/100PY], 95% confidence interval [CI]: 0.8-2.3). The second and third most common types contributing to persistent infection were HPV52 (IR: 1.2 /100PY, 95% CI: 0.6-2.1) and HPV39 (IR: 1.1 /100PY, 95% CI: 0.6-1.9), respectively. These types were followed by HPV45 (IR: 0.9 /100PY, 95% CI: 0.4-1.7) and HPV35 (IR: 0.7 /100PY, 95% CI: 0.3-1.4) being fourth and fifth most common, respectively. Overall, 40% of persistent infections were cases in which the HPV type was detected in at least two consecutive samples while HPV was detected in the last sample in 60% of cases. This 40%/60% split between confirmed persistent and last sample cases was also consistent within HPV types.

In a sensitivity analysis that limited to only the confirmed persistent cases (not including cases of HPV detection in the last sample), the most frequently documented HPV type remained as HPV51 (IR: 0.6 /100PY, 95% CI: 0.2-1.2), followed by HPV52 (IR: 0.5 /100PY, 95% CI: 0.2-1.1) and HPV39 (IR: 0.4 /100PY, 95% CI: 0.1-1.0), respectively.
In a sub-analysis pooling HPV types into categories of nonavalent (HPV31/33/45/52/58) or oncogenic HPV types not contained within available vaccines (HPV35/39/51/56/59/68), the composite endpoints yielded an incidence rate of 2.4 /100PY (95% CI: 1.6-3.5) for persistent infection with nonavalent HPV types and an incidence rate of 3.6 /100PY (95% CI: 2.6-4.9) for persistent infection with HPV types not contained within vaccines.

### 7.4 Discussion

Of the top five persistent HPV types observed in this cohort, only HPV52 and 45 are contained within the nonavalent vaccine. Additionally, the persistent infection with the HPV types added in the nonavalent vaccine that are not present in the quadrivalent vaccine had an incidence rate of 2.4 /100PY while persistent infection with HPV types contained within any available vaccine resulted in a higher incidence rate of 3.6 /100PY. This implies that the nonavalent vaccine could further assist in the protection of WLWH, but gaps in protection for this population would remain. Although the HPV types that are not contained within any currently available vaccine contribute less to disease in the general population, they are carcinogenic and the effect of HIV infection on the pathogenicity of these specific HPV types has not been completely elucidated. Description of HPV types associated with CIN3+ in women without HIV and WLWH has shown that the contribution of HPV51 and 39 towards dysplasia in WLWH is greater than in women without HIV (127). Meta analysis has also shown that WLWH who have HSIL are less likely to be infected with HIV16 than the general population and more likely to be infected with HPV51, among other types, or have multiple HPV type infection (124). HIV is known to disrupt epithelial tight junctions, which may facilitate HPV entry to the basal epithelial layer (54). It is also known that the HIV tat protein enhances HPV transcription (126). This could be a mechanism explaining the potential oncogenic effects of HPV genotypes that could differentially affect WLWH. As the HPV types not contained within available vaccines may cause disease in this way, it is likely that the infectivity and carcinogenic potential of these HPV types is enhanced in WLWH.

Further study is needed to more clearly describe the contribution of these HPV types to cervical dysplasia among WLWH.

The high rate of persistent infection with HPV51 validates previous data indicating that there is a high burden of HPV51 in WLWH and that this type would be very important in WLWH post-vaccination (162). We observed less persistent HPV31 and HPV33 than reported in some previous studies of North American WLWH (53, 124). However, we did see relatively high rates of persistent HPV52 and HPV58, which is consistent with prior literature in WLWH (124). We might hypothesize the differences could be a result of some cross-protection against HPV31, which is closely related to HPV16 within the alpha-9 phylogenetic group, and HPV33, which is also an alpha-9 HPV type. Evidence of cross-protection against HPV31 and HPV33 by the quadrivalent HPV vaccine has previously been documented (163).

The main analysis was conservative in nature and provides an overestimate of the incidence of persistent infection as not all cases of HPV detected at the last visit will go on to truly persist. The sensitivity analysis provided the opposite scenario of an underestimate of persistent infection as it only included cases where the HPV type was documented at two consecutive visits. Taken together, these analyses were consistent in demonstrating that HPV51, 52, and 39 contribute the largest burden of persistent infection among this vaccinated population, and they demonstrate the upper and lower limits within which the true value of persistent infection lies.

To our knowledge, only one other paper has described HPV infection with non-vaccine HPV types post-vaccination within a population of WLWH, but women were only followed for one year post-vaccination (164). Similar to our findings, they reported a higher

frequency of the non-vaccine HPV types 51 and 52 detected at 28 and 52 weeks. In contrast to our findings, they detected a relatively high frequency of HPV31 at the 52-week time point and HPV68 at both time points, but not a higher frequency of HPV39 (164).

### 7.4.1 Conclusions

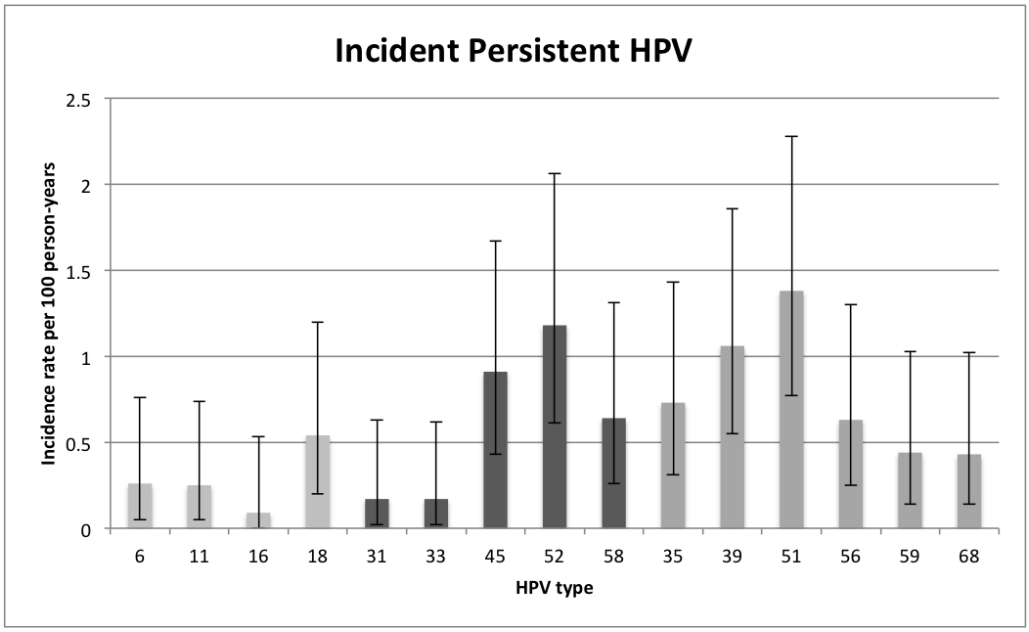
Our findings add critical data to the literature regarding persistent HPV infection with extended follow-up post-vaccination in WLWH. WLWH who have been vaccinated with the quadrivalent HPV vaccine remain vulnerable to a clinically significant burden of persistent HPV infections. The frequency with which these strains lead to cervical dysplasia and cancer requires ongoing study. Although the nonavalent vaccine has the potential to eliminate a portion of that burden, many of the persistent HPV infections that WLWH face are due to HPV types not contained within any currently available vaccine. Our findings support the continued regular cervical screening of WLWH regardless of their HPV vaccine history and validate the need for a multipronged approach to eradication of cervical cancer.

**Table 7.1** Study Population Characteristics (n=284)

| Characteristic | N (%) or Median (IQR) |
|---|---|
| Age | 38 (32-44) |
| Ethnicity | |
| Black | 117 (41%) |
| White | 103 (36%) |
| Indigenous | 41 (14%) |
| Other | 23 (8%) |
| Baseline CD4 count (cells/$mm^3$) | 499 (375-680) |
| CD4 nadir (cells/$mm^3$) | 230 (120-338) |
| HIV viral load suppression (VL<50 copies/ml) | 195 (71%) |
| Probable mode of HIV acquisition | |
| Blood products | 12 (4%) |
| Injection drug use | 45 (16%) |
| Mother-to-child | 12 (4%) |
| Sexual contact | 185 (65%) |
| Other | 30 (11%) |
| Number of vaccine doses received | |
| 3 | 267 (94%) |
| 2 | 9 (3%) |
| 1 | 8 (3%) |

Abbreviations: IQR, interquartile range.

**Figure 7.1** Incident Persistent HPV




Light grey: quadrivalent HPV types; dark grey: additional HPV types in the nonavalent vaccine; medium grey: oncogenic HPV types not contained within available vaccines

# CHAPTER 8: SUMMARY, CONTRIBUTION, FUTURE RESEARCH, AND CONCLUSION

## 8.1 Summary of Findings

The research described herein has made a substantial impact on the global literature regarding HPV vaccination in girls and women living with HIV (WLWH). At the initiation of this thesis work, there was little literature describing HPV vaccine immunogenicity in girls and WLWH and no published data on vaccine efficacy, dose spacing of the HPV vaccine, or residual burden of non-vaccine oncogenic HPVs in this population. Additionally, the existing literature on HPV type prevalence and persistence in a population of WLWH in Canada had become outdated. This work was timely given the 2019 announcement of the World Health Organization (WHO) global call to action for cervical cancer elimination (106). Reaching elimination of cervical cancer, particularly in a rapid fashion, will require a focus on WLWH who bear a disproportionate burden of cervical cancer. This thesis fills important gaps and informs the WHO cervical cancer elimination strategy using data from an ongoing national cohort study of quadrivalent HPV vaccination in girls and WLWH in Canada.

Chapter 4, Prevalent and Persistent Oncogenic HPV Types in a Cohort of Women Living with HIV Prior to HPV Vaccination, in revisions in 2020, provides a description of the prevalence, persistence, and predictors of oncogenic HPV infection in WLWH in Canada prior to HPV vaccination. It also describes cases of high-grade cervical cytology and the associated HPV types in this population. Our findings indicate that almost half of the cohort (45.2%) was infected with at least one oncogenic HPV type and that one third of participants had a persistent oncogenic HPV infection between the two visits analyzed. These cases of

prevalent and persistent HPV infection were caused by a wide range of oncogenic HPV types. Importantly, cases of persistent oncogenic HPVs not contained within any available vaccine were associated with low CD4 counts, suggesting that treatment of HIV in order to achieve immune reconstitution is important for HPV-associated cancer prevention. Although there were only seven cases of cytological HSIL present at the baseline visit, only at most two (29%) of these cases were associated with HPV16 infection while up to four (57%) of these cases were associated with other oncogenic HPV types not contained within any currently available vaccine. These findings highlight the importance of optimal HIV treatment and underscore the need for continued diligent cervical cancer screening post-vaccination as key steps towards global elimination of cervical cancer.

Chapter 5, Immunological Impact of Quadrivalent HPV Vaccine Dose Spacing in Women Living with HIV, published in *Vaccine* in 2020, assesses the impact of differential dose spacing of the quadrivalent HPV vaccine on vaccine immunogenicity in WLWH (117). Although we were unable to assess the impact of one or two doses of vaccine on the vaccine immunogenicity due to low numbers of women in our cohort who received less than three doses (n=10), we were able to analyze differential spacing of the three vaccine doses to produce novel data on the impact of spacing which will inform global vaccine recommendations for WLWH. Participants were divided into groups based on their vaccine spacing: three doses of vaccine within 7 months (n=229), three doses within seven months to one year (n=56), and three doses within one to two years (n=22). The analysis demonstrates that when age, time to serology, CD4 cell count, CD4 nadir, HIV viral load, and HPV naivety are all built into the model, the spacing of the three quadrivalent HPV vaccine doses does not significantly impact peak HPV antibody titers. Additionally, in corroboration with

our previous findings, there is a relationship between the peak HPV antibody response and HIV viral load (105). An expected relationship between HPV type naivety and peak antibody response, as well as time to serology after vaccination and peak antibody response is present. These findings are critical to vaccine programming and provision globally as they indicate that there is no evidence to suggest that restarting the vaccine schedule or adding an additional dose in these situations of expanded dose intervals is necessary.

Chapter 6, The Efficacy of the Quadrivalent Human Papillomavirus Vaccine in Girls and Women Living with HIV, published in *Clinical Infectious Diseases* in 2019 (118), provides the first published data on HPV vaccine efficacy data in women living with HIV. At two years post vaccination in the per-protocol efficacy group, the incidence of persistent quadrivalent HPV infection is 1.0 per 100 person-years, the incidence of genital warts is 1.0 per 100 person-years, and there are no cases of qHPV-associated CIN2+ in women with normal baseline cervical cytology. Based on comparison to a group of vaccinated women without HIV, these findings demonstrate that vaccinated WLWH may be at higher risk for vaccine failure because the incidence of endpoints is similar to the HIV-negative placebo group. However, overall rates of vaccine failure are low and rates of persistent quadrivalent HPV are lower than the rates previously seen in unvaccinated WLWH. Although the numbers of individuals with persistent HPV infection or genital warts were too low for robust statistical analysis, baseline CD4 count and CD4 nadir appear to be lower among cases, suggesting that present and historical immune compromise may be important predictors of vaccine failure. These findings are a very important contribution to the literature and longer follow-up on these endpoints will be critical to further informing HPV vaccination practices for WLWH.

Chapter 7, Persistence of Non-Vaccine Oncogenic HPV Genotypes in Quadrivalent HPV-Vaccinated Women Living with HIV, published in the *Journal of Acquired Immune Deficiency Syndromes* in 2020, assesses the rates of persistent infection with oncogenic HPV types not contained in the quadrivalent HPV vaccine in quadrivalent HPV-vaccinated WLWH in Canada (165). The findings are important because they describe the burden of persistent oncogenic HPV infection that quadrivalent HPV-vaccinated WLWH continue to face and suggest which HPV types may contribute most to cervical precancerous lesions post-vaccination in this population. The most common types contributing to persistent infection were HPV51, 52, and 39 with incidence rates of 1.4 (n=15, CI: 0.8-2.3), 1.2 (n=12, CI: 0.6-2.1), and 1.1 (n=12, CI: 0.6-1.9) per 100 person-years, respectively. While the nonavalent vaccine could alleviate some of this burden, two of these top three persistent oncogenic HPVs in this cohort are not contained within any available vaccine. Additionally, when HPV types were pooled, the types contained in the nonavalent vaccine contributed less to persistent infection than the types that are not contained within any available vaccine. These findings emphasize the need for ongoing cervical screening in HPV-vaccinated WLWH.

Taken together, the data presented in this thesis describe aspects of HPV vaccination in WLWH from pre to post-vaccination stages and serve to inform global vaccination strategies including the WHO cervical cancer elimination strategy. The evidence of a need for HPV vaccination in WLWH, beyond the current adolescent-based programs, is clear and supported by high rates of pre-vaccination HPV persistence. We show that the spacing of the recommended three vaccine doses does not significantly impact the peak antibody titer achieved in our cohort, and therefore there is no evidence to suggest that spacing of the three

doses up to two years apart warrants restarting the vaccine schedule or providing a booster dose. Once provided with the vaccine, we find the efficacy at two years post-vaccination to be good, with vaccinated women having low rates of persistent HPV infection, genital warts, and no cases of CIN2+. However, the efficacy is lower than that seen in a cohort of women without HIV and, therefore, further follow-up is ongoing. Post-vaccination, persistence of HPV types not contained in the vaccine was quantified and rates of persistent infection with a number of HPV types are not significantly different from pre-vaccination rates of persistent HPV18 in WLWH, emphasizing the ongoing role of cervical cancer screening in this vulnerable population.

### 8.2 Limitations

The size of the cohort described in this thesis was moderate but represents WLWH from multiple provinces and sites of HIV care across Canada. As recruitment took place in this setting where the population was generally well engaged in care, our findings may not be generalizable to other settings. In these analyses, baseline CD4 count and HIV viral load were generally assessed as predictors of outcomes of interest; it is important to note that these measures are often not constant over time and therefore an individual's CD4 count or HIV viral load may have changed prior to the outcome of interest. Further analyses incorporating longitudinal measures of these parameters will be pursued. Due to the high level of engagement in care, the vast majority of participants received all three doses of vaccine, which rendered us unable to assess the comparative immunogenicity to one or two doses. The efficacy analysis in this thesis was limited to two years of follow-up, which is not adequate to fully elucidate the efficacy of the vaccine. However, follow-up for this cohort

will extend up to eight years post-vaccination. Further follow-up will also address the limitation of the shorter follow-up time with respect to the development of CIN2+, which often takes longer than two years to develop. Due to the ethical limitation of not using a placebo group, we made comparisons to published data from a cohort of women without HIV and a historical cohort of women with HIV. It is important to note that the vaccine failures identified (i.e. reaching efficacy endpoints of new persistent qHPV, new genital warts, or new qHPV associated CIN2+) are not necessarily true vaccine failures. It is possible that partner deposition of HPV is responsible for detection of some HPV cases and that some HPV was undetected at the screening and baseline visits. Low rates of vaccine failure precluded our ability to assess predictors of such events. Additionally, it should be noted that the endpoints of persistent infection and CIN2+ used for this study are used as they are nearest surrogates for the true endpoint of interest, which is invasive cervical cancer. However, only a percentage of these cases would become cervical cancer and the distribution of HPV types in cervical cancer cases likely would not exactly match the distribution of HPV types in persistent infections. Finally, low rates of cytological HSIL at baseline prevented us from determining the attributable risk for each oncogenic HPV type.

### 8.3 Unique Contributions

Many of the unique contributions of this work stem from its novelty and global impact. At the time of thesis initiation, there was very little data on HPV vaccination in WLWH globally, and the published literature that did exist primarily assessed immunogenicity to the vaccine. Despite this lack of data, the HPV vaccine had been implemented in many global settings including those with significant proportions of WLWH.

The vaccine was known to be safe and therefore it was appropriate to offer it to WLWH without delay; however, the need for data to evaluate the vaccine in this population was urgent. This urgency stemmed from the known importance of HPV prevention in WLWH due to their higher risk of disease as well as the knowledge that other vaccines do not function as well in immunocompromised individuals. While the existing HPV vaccine immunogenicity data for WLWH was very important, efficacy outcomes are of greater importance as they more directly relate to the clinical outcomes (precancer and cancer) that have a real impact on patients. The findings described in Chapter 6 were the first published HPV vaccine efficacy findings globally in WLWH, and therefore represent an important and unique contribution to the literature.

Chapters 5 and 7 also contribute novel information to the literature as the first studies reporting outcomes of differential dose spacing for the HPV vaccine in WLWH and the HPV types that contributed to persistent oncogenic HPV infection beyond one year post-vaccination. Understanding the impact of dose spacing for the HPV vaccine is vital to inform management decisions where the suggested spacing of the vaccine doses cannot be achieved due to system or patient factors. This is of particular importance in cases where women or countries are unable to pay for additional doses of vaccine if the schedule is interrupted. The unique contribution of novel data on post-vaccination HPV persistence is also essential to inform cervical screening strategies in WLWH post-vaccination.

Additional strengths and contributions of this work stem from the fact that this thesis utilizes data from a pan-Canadian cohort of WLWH. Recruiting women from across the country allowed us not only to have adequate numbers to assess the primary study outcomes, but also to have a more current and representative cohort of Canadian WLWH. Time and

geographic trends in HPV prevalence exist, and therefore updates in country-specific data on HPV prevalence are required. The prior national study of HPV in WLWH from the early 2000's had become outdated. Hence, we began in Chapter 4 by describing the pre-vaccination rates of HPV prevalence and persistence in the cohort. Having this baseline snapshot of a pan-Canadian cohort of WLWH will surely be useful in future studies assessing the ongoing impact of HPV vaccination in this population.

A unique output resulting from this thesis work was the publication of cervical cancer screening guidelines for immunocompromised women, published in the *Journal of Obstetrics and Gynecology Canada* in 2019 (166). The need for such guidance had become increasingly evident, as many changes to cervical cancer screening programs had come to pass in the previous five years, with no detailed accompanying guidance for WLWH and other immunocompromised women due to a lack of data for this population. Additionally, cervical screening recommendations varied between provinces, further muddying the waters in terms of what care providers for immunocompromised women should do. We felt that it was important to use the information we were gathering from the HPV in HIV Study to help inform cervical screening recommendations for this group of women. The resulting publication collates the existing background data and considers the resource impact and potential harms before providing clear recommendations for screening in immunocompromised women across Canada.

### 8.4 Future Research

The field of HPV vaccination has rapidly evolved over the duration of the study described herein. Important changes have included the introduction of the nonavalent HPV

vaccine as well as reduced dose schedules for individuals aged 9-14, with the possibility of a one-dose schedule now being explored. As these changes occurred, we created relevant research outputs by separating out the added nonavalent HPV types when appropriate but were unable to assess the impact of the reduced dose schedules due to high adherence to the three recommended doses of vaccine in our cohort. Future research to explore the use of the nonavalent vaccine and the concept of reduced dosing in WLWH is of utmost importance, particularly as these reduced-dose schedules are implemented in global settings, including HIV-endemic countries, without evidence to support their use in girls and WLWH.

Extended follow-up of the existing cohort is also an important future direction for research. Immunogenicity and efficacy data beyond two years of follow-up is required to reach more definitive conclusions regarding efficacy against clinical endpoints of infection and disease, which often take many years to develop. With the development of CIN2+ cases, the attribution of HPV types responsible for these cases will also provide important information not only for vaccine recommendations, but also to inform HPV-based screening. As further data is collected from this cohort of quadrivalent HPV-vaccinated WLWH, the data to suggest whether booster dosing is required for this population may become apparent. If seropositivity rates decline significantly and/or vaccine failures are demonstrated, a booster dose of HPV vaccine should be explored. Use of either a quadrivalent or nonavalent HPV vaccine dose as a booster will allow for assessment of whether or not there is an anamnestic response to the quadrivalent HPV genotypes. Immunizing with the nonavalent vaccine would still provide information regarding the presence of an anamnestic response while also providing protection against the added nonavalent HPV types as well as limited one-dose data on those HPV types, depending on the immunization schedule used.

Additional studies to explore HPV vaccine outcomes among girls and WLWH are critically needed in other regions of the world. HIV-endemic regions often do not have access to organized cervical cancer screening programs, resulting in the highest rates of cervical cancer deaths in these regions. Therefore, these regions stand to benefit the most from HPV vaccination. The cohort described herein was a highly engaged cohort of females seeking specialized HIV care in Canada, and with access to a range of free antiretroviral therapy options. Regions of the world that are HIV-endemic or have reduced access to HIV care and antiretroviral therapy require their own data to assess the efficacy of the HPV vaccine in their settings, as important differences may be present.

Research that deeper explores the immunology side of HPV vaccination in WLWH could shed light on many remaining questions. Within the first two years of follow-up, the efficacy that we observed in our cohort of WLWH was not as great as that seen in vaccinated women without HIV, despite high immune titers achieved in these women. If longer follow-up of these participants continues to demonstrate reduced efficacy among WLWH, studies to investigate the functionality of the anti-HPV antibody response created by the HPV vaccine could hold the explanation for this observation. Immunological studies could also begin to clarify the relationship between HIV viral load and peak anti-HPV antibody response, as HIV viral load was an important predictor of peak antibody response in the HPV in HIV cohort.

Qualitative studies of HPV vaccination in WLWH would contribute greatly to the literature and will be beneficial to conduct in a variety of settings. For example, the availability of an HPV vaccine does not mean that all girls and WLWH will receive it; understanding the acceptability of the HPV vaccine in populations of girls and WLWH from various global settings can inform strategies to ensure that as many people as possible receive

the vaccine and are protected against vaccine-containing HPV types. Mixed methods or qualitative studies could also explore the uptake of HPV vaccination in girls living with HIV, compared to that among girls without HIV. If a difference in uptake is present, qualitative methodologies can help to determine barriers or enablers to HPV vaccination that are specific to girls and WLWH.

Given the rapidly evolving nature of the HPV vaccination space and the lack of data to date specifically for girls and WLWH, the future directions for research in this area abound.

### 8.5 Conclusion

This thesis represents a novel and important contribution to the literature on HPV vaccination. It describes key aspects of HPV vaccination in WLWH from the pre-vaccination to post-vaccination time points and informs the WHO global strategy for cervical cancer elimination. Pre-vaccination rates of prevalent and persistent oncogenic HPV infection were high, supporting the need for HPV vaccination in this population. We demonstrated that the spacing of the three vaccine doses did not significantly impact the peak anti-HPV antibody titer achieved, which suggests that extending the spacing interval between the first and third doses up to two years should not warrant re-initiation of the vaccine schedule. At two years post-vaccination, the efficacy of the vaccine was good, demonstrating lower rates of clinical endpoints than previously documented in Canadian WLWH who had not been vaccinated. However, the protection of the vaccine was not as complete as that seen in vaccinated women without HIV. Following vaccination, we also quantified the persistence of non-vaccine oncogenic HPV types, finding higher rates of persistent infection with non-vaccine HPV

types versus HPV types covered by the nonavalent vaccine. This emphasizes the need for ongoing cervical cancer screening post-vaccination in WLWH. Collectively, these findings have provided great value to the clinical care of WLWH by informing best vaccination and screening practices for this particularly vulnerable population. Continued follow-up of these women, as well as future quantitative and qualitative studies in various populations of WLWH, will provide needed further data on HPV vaccination and screening in WLWH.

## BIBLIOGRAPHY


1. Cullen TS. Cancer of the uterus; its pathology, symptomatology, diagnosis, and treatment. United States. eBook1900.

2. Reagan JW, Seidemann IL, Saracusa Y. The cellular morphology of carcinoma in situ and dysplasia or atypical hyperplasia of the uterine cervix. Cancer. 1953;6(2):224.

3. Richart RM, Barron BA. A follow-up study of patients with cervical dysplasia. American Journal of Obstetrics and Gynecology. 1969;105(3):386.

4. Robertson AJ, Anderson JM, Beck JS, Burnett RA, Howatson SR, Lee FD, et al. Observer variability in histopathological reporting of cervical biopsy specimens. Journal of Clinical Pathology. 1989;42(3):231-8.

5. Ismail SM, Colclough AB, Dinnen JS, Eakins D, Evans DMD, Gradwell E, et al. Reporting cervical intra-epithelial neoplasia (CIN): Intra- and interpathologist variation and factors associated with disagreement. Histopathology. 1990;16(4):371-6.

6. The 1988 Bethesda System for Reporting Cervical/Vaginal Cytological Diagnoses. JAMA. 1989;262(7):931-4.

7. Richart RM. A Modified Terminology for Cervical Intraepithelial Neoplasia. Obstetrics & Gynecology. 1990;75(1):131-3.

8. Darragh TM, Colgan TJ, Thomas Cox J, Heller DS, Henry MR, Luff RD, et al. The Lower Anogenital Squamous Terminology Standardization Project for HPV-associated Lesions: Background and Consensus Recommendations From the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. International Journal of Gynecological Pathology. 2013;32(1):76-115.

9. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018;68(6):394-424.

10. Canadian Cancer Society. Canadian Cancer Statistics 2014. Toronto; 2014.

11. Sankaranarayanan R, Ferlay J. Worldwide burden of gynaecological cancer: The size of the problem. Best Practice & Research Clinical Obstetrics & Gynaecology. 2006;20(2):207-25.

12. Ferlay J EM, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F. Global Cancer Observatory: Cancer Today Lyon, France: International Agency for Research on Cancer; 2018 [10 December 2019]. Available from: https://gco.iarc.fr/today.

13. International Agency for Research on Cancer. Human Papillomaviruses - IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Geneva: World Health Organization; 2007.

14. Saraiya M, Unger ER, Thompson TD, Lynch CF, Hernandez BY, Lyu CW, et al. US Assessment of HPV Types in Cancers: Implications for Current and 9-Valent HPV Vaccines. JNCI. 2015;107(6):1.

15. Brickman C, Palefsky JM. Human Papillomavirus in the HIV-Infected Host: Epidemiology and Pathogenesis in the Antiretroviral Era. Current HIV/AIDS Reports. 2015;12(1):6-15.

16. de Villiers EM. Cross-roads in the classification of papillomaviruses. Virology. 2013;445(1-2):2-10.

17. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic Classification of Human Papillomavirus Types Associated with Cervical Cancer. The New England Journal of Medicine. 2003;348(6):518-27.

18. Muñoz N, Castellsagué X, de González AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. Vaccine. 2006;24:S1-S10.

19. Sellors JW, Mahony JB, Kaczorowski J, Lytwyn A, Bangura H, Chong S, et al. Prevalence and predictors of human papillomavirus infection in women in Ontario, Canada. Survey of HPV in Ontario Women (SHOW) Group. CMAJ. 2000;163.

20. Ratnam S, Franco EL, Ferenczy A. Human Papillomavirus Testing for Primary Screening of Cervical Cancer Precursors. Cancer Epidemiology Biomarkers & Prevention. 2000;9(9):945.

21. Smith JS, Melendy A, Rana RK, Pimenta JM. Age-Specific Prevalence of Infection with Human Papillomavirus in Females: A Global Review. Journal of Adolescent Health. 2008;43(4):S5.e1-S5.e62.

22. Mosavi-Jarrahi AM, Kliewer EV. Cervical Cancer Incidence Trends in Canada: A 30-Year Population-Based Analysis. Journal of Obstetrics and Gynaecology Canada. 2013;35(7):620-6.

23. Husain RSA, Ramakrishnan V. Global Variation of Human Papillomavirus Genotypes and Selected Genes Involved in Cervical Malignancies. Annals of Global Health. 2015;81(5):675-83.

24. Patel H, Wagner M, Singhal P, Kothari S. Systematic review of the incidence and prevalence of genital warts. BMC Infectious Diseases. 2013;13(1):1-14.

25. Koutsky PL. Epidemiology of Genital Human Papillomavirus Infection. The American Journal of Medicine. 1997;102(5):3-8.

26. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine. 2006;24:S4-S15.

27. Dareng EO, Adebamowo SN, Famooto A, Olawande O, Odutola MK, Olaniyan Y, et al. Prevalence and incidence of genital warts and cervical Human Papillomavirus infections in Nigerian women. BMC Infectious Diseases. 2019;19(1):27.

28. Bryan JT. Developing an HPV vaccine to prevent cervical cancer and genital warts. Vaccine. 2007;25(16):3001-6.

29. von Krogh G, Lacey CJN, Gross G, Barrasso R, Schneider A. European course on HPV associated pathology: guidelines for primary care physicians for the diagnosis and management of anogenital warts. Sexually Transmitted Infections. 2000;76(3):162-8.

30. Maw RD, Reitano M, Roy M. An international survey of patients with genital warts: perceptions regarding treatment and impact on lifestyle. Int J STD AIDS. 1998;9.

31. Aksoy P, Gottschalk EY, Meneses PI. HPV entry into cells. Mutation Research-Reviews in Mutation Research. 2017;772:13-22.

32. Vonsky M, Shabaeva M, Runov A, Lebedeva N, Chowdhury S, Palefsky JM, et al. Carcinogenesis Associated with Human Papillomavirus Infection. Mechanisms and Potential for Immunotherapy. Biochemistry. 2019;84(7):782-99.

33. Stanley MA. Epithelial Cell Responses to Infection with Human Papillomavirus. Clinical Microbiology Reviews. 2012;25(2):215-22.

34. Tindle RW. Immune evasion in human papillomavirus-associated cervical cancer. Nature Reviews Cancer. 2002;2(1):59-64.

35. Lee SJ, Cho YS, Cho MC, Shim JH, Lee KA, Ko KK, et al. Both E6 and E7 oncoproteins of human papillomavirus 16 inhibit IL-18-induced IFN-gamma production in human peripheral blood mononuclear and NK cells. Journal of Immunology. 2001;167(1):497-504.

36. Guess JC, McCance DJ. Decreased Migration of Langerhans Precursor-Like Cells in Response to Human Keratinocytes Expressing Human Papillomavirus Type 16 E6/E7 Is Related to Reduced Macrophage Inflammatory Protein-3α Production. Journal of Virology. 2005;79(23):14852-62.

37. DiMaio D, Petti LM. The E5 proteins. Virology. 2013;445(1):99-114.

38. Venuti A, Paolini F, Nasir L, Corteggio A, Roperto S, Campo MS, et al. Papillomavirus E5: the smallest oncoprotein with many functions. Molecular Cancer. 2011;10(1):140.

39. Hengstermann A, Linares LK, Ciechanover A, Whitaker NJ, Scheffner M. Complete Switch from Mdm2 to Human Papillomavirus E6-Mediated Degradation of p53 in Cervical Cancer Cells. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(3):1218-23.

40. Dyson NJ. RB1: a prototype tumor suppressor and an enigma. Genes & Development. 2016;30(13):1492-502.

41. Vink MA, Bogaards JA, van Kemenade FJ, de Melker HE, Meijer CJLM, Berkhof J. Clinical progression of high-grade cervical intraepithelial neoplasia: estimating the time to preclinical cervical cancer from doubly censored national registry data. American Journal of Epidemiology. 2013;178(7):1161-9.

42. Gupta S, Kumar P, Das BC. HPV: Molecular pathways and targets. Current Problems in Cancer. 2018;42(2):161-74.

43. Holmes A, Lameiras S, Jeannot E, Marie Y, Castera L, Sastre-Garau X, et al. Mechanistic signatures of HPV insertions in cervical carcinomas. NPJ Genomic Medicine. 2016;1(1):16004.

44. Hu Z, Zhu D, Wang W, Li W, Jia W, Zeng X, et al. Genome-wide profiling of HPV integration in cervical cancer identifies clustered genomic hot spots and a potential microhomology-mediated integration mechanism. Nature Genetics. 2015;47(2):158-63.

45. Oyervides-Muñoz MA, Pérez-Maya AA, Rodríguez-Gutiérrez HF, Gómez-Macias GS, Fajardo-Ramírez OR, Treviño V, et al. Understanding the HPV integration and its progression to cervical cancer. Infection, Genetics and Evolution. 2018;61:134-44.

46. Anderson GH, Boyes DA, Benedet JL, Le Riche JC, Matisic JP, Suen KC, et al. Organisation and results of the cervical cytology screening programme in British Columbia, 1955-85. British Medical Journal. 1988;296(6627):975-8.

47. Shaw P. The History of Cervical Screening I: The Pap. Test. The Journal of Obstetrics and Gynecology Canada. 2000;22(2):110-14.

48. Stuart GC, McGregor SE, Duggan MA, Nation JG. Review of the screening history of Alberta women with invasive cervical cancer. Canadian Medical Association Journal. 1997;157(5):513-9.

49. Ogilvie GS, Krajden M, van Niekerk D, Smith LW, Cook D, Ceballos K, et al. HPV for cervical cancer screening (HPV FOCAL): Complete Round 1 results of a randomized trial comparing HPV-based primary screening to liquid-based cytology for cervical cancer. International Journal of Cancer. 2017;140(2):440-8.

50. Ronco GD, Dillner JP, Elfström KMMPH, Tunesi SP, Snijders PJFP, Arbyn MMD, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet. 2014;383(9916):524-32.

51. Guidry JT, Scott RS. The interaction between human papillomavirus and other viruses. Virus Research. 2017;231:139-47.

52. Luque AE, Jabeen M, Messing S, Lane CA, Demeter LM, Rose RC, et al. Prevalence of Human Papillomavirus Genotypes and Related Abnormalities of Cervical Cytological Results among HIV-1-Infected Women in Rochester, New York. The Journal of Infectious Diseases. 2006;194(4):428-34.

53. Massad LS, Xie X, Burk R, Keller MJ, Minkoff H, D'Souza G, et al. Long-term cumulative detection of human papillomavirus among HIV seropositive women. AIDS. 2014;28(17):2601-8.

54. Tugizov SM, Herrera R, Chin-Hong P, Veluppillai P, Greenspan D, Michael Berry J, et al. HIV-associated disruption of mucosal epithelium facilitates paracellular penetration by human papillomavirus. Virology. 2013;446(1-2):378-88.

55. Nazli A, Chan O, Dobson-Belaire WN, Ouellet M, Tremblay MJ, Gray-Owen SD, et al. Exposure to HIV-1 Directly Impairs Mucosal Epithelial Barrier Integrity Allowing Microbial Translocation. PLOS Pathogens. 2010;6(4):e1000852.

56. van der Burg SH, Palefsky JM. Human Immunodeficiency Virus and Human Papilloma Virus - why HPV-induced lesions do not spontaneously resolve and why therapeutic vaccination can be successful. Journal of Translational Medicine. 2009;7(1):108-.

57. Kang M, Cu-Uvin S. Association of HIV Viral Load and CD4 with HPV Detection and Clearance in HIV Infected Women Initiating HAART. HIV Medicine. 2012;13(6):372-8.

58. Branca M, Costa S, Mariani L, Sesti F, Agarossi A, di Carlo A, et al. Assessment of risk factors and human papillomavirus (HPV) related pathogenetic mechanisms of CIN in HIV-positive and HIV-negative women. Study design and baseline data of the HPV-PathogenISS study. European Journal of Gynaecological Oncology. 2004;25(6):689.

59. Ferenczy A, Coutlee F, Franco E, Hankins C. Human papillomavirus and HIV coinfection and the risk of neoplasias of the lower genital tract: a review of recent developments. Canadian Medical Association Journal. 2003;169(5):431-4.

60. Grulich AE, Van Leeuwen MT, Falster MO. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet. 2007;370:59.

61. Adler DH, Wallace M, Bennie T, Mrubata M, Abar B, Meiring TL, et al. Cervical Dysplasia and High-Risk Human Papillomavirus Infections among HIV-Infected and HIV-Uninfected Adolescent Females in South Africa. Infectious Diseases in Obstetrics and Gynecology. 2014;2014:1-6.

62. Massad LS, Riester KA, Anastos KM, Fruchter RG. Prevalence and predictors of squamous cell abnormalities in papanicolaou smears from women infected with HIV-1. Journal of Acquired Immune Deficiency Syndromes. 1999;21(1):33.

63. Duerr A, Kieke B, Warren D, Shah K, Burk R, Peipert JF, et al. Human papillomavirus–associated cervical cytologic abnormalities among women with or at risk of infection with human immunodeficiency virus. American Journal of Obstetrics and Gynecology. 2001;184(4):584-90.

64. Strickler HD, Burk RD, Fazzari M, Anastos K, Minkoff H, Massad LS, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. Journal of the National Cancer Institute. 2005;97(8):577-86.

65. Denslow SA, Rositch AF, Firnhaber C, Ting J, Smith JS. Incidence and progression of cervical lesions in women with HIV: a systematic global review. London, England: SAGE Publications; 2014. p. 163-77.

66. Parham GP, Sahasrabuddhe VV, Mwanahamuntu MH, Shepherd BE, Hicks ML, Stringer EM, et al. Prevalence and predictors of squamous intraepithelial lesions of the cervix in HIV-infected women in Lusaka, Zambia. Gynecologic Oncology. 2006;103(3):1017-22.

67. Dolev JC, Maurer T, Springer G, Glesby MJ, Minkoff H, Connell C, et al. Incidence and risk factors for verrucae in women. AIDS. 2008;22(10):1213-9.

68. Silverberg MJ, Ahdieh L, Munoz A, Anastos K, Burk RD, Cu-Uvin S, et al. The impact of HIV infection and immunodeficiency on human papillomavirus type 6 or 11 infection and on genital warts. Sexually Transmitted Diseases. 2002;29(8):427-35.

69. De Panfilis G, Melzani G, Mori G, Ghidini A, Graifemberghi S. Relapses after treatment of external genital warts are more frequent in HIV-positive patients than in HIV-negative controls. Sexually Transmitted Diseases. 2002;29(3):121-5.

70. Negin J, Aspin C, Gadsden T, Reading C. HIV Among Indigenous peoples: A Review of the Literature on HIV-Related Behaviour Since the Beginning of the Epidemic. AIDS and Behavior. 2015;19(9):1720-34.

71. Public Health Agency of Canada. Summary: Estimates of HIV Incidence, Prevalence and Canada's Progress on Meeting the 90-90-90 HIV Targets, 2016. Ottawa; 2018.

72. Moore SPD, Antoni SMPH, Colquhoun AM, Healy BRN, Ellison-Loschmann LP, Potter JDP, et al. Cancer incidence in indigenous people in Australia, New Zealand, Canada, and the USA: a comparative population-based study. Lancet Oncology. 2015;16(15):1483-92.

73. Lawton B, Heffernan M, Wurtak G, Steben M, Lhaki P, Cram F, et al. IPVS Policy Statement addressing the burden of HPV disease for Indigenous peoples. Papillomavirus Research. 2020;9:100191.

74. Jiang Y, Brassard P, Severini A, Mao Y, Li YA, Laroche J, et al. The prevalence of human papillomavirus and its impact on cervical dysplasia in Northern Canada. Infect Agent Cancer. 2013;8(1):25.

75. Healey SM, Aronson KJ, Mao Y, Schlecht NF, Mery LS, Ferenczy A, et al. Oncogenic human papillomavirus infection and cervical lesions in aboriginal women of Nunavut, Canada. Sexually Transmitted Diseases. 2001;28(12):694-700.

76. Young TK, Kliewer E, Blanchard J, Mayer T. Monitoring disease burden and preventive behavior with data linkage: cervical cancer among aboriginal people in Manitoba, Canada. American Journal of Public Health. 2000;90(9):1466-8.

77. Zehbe I, Moeller H, Severini A, Weaver B, Escott N, Bell C, et al. Feasibility of self-sampling and human papillomavirus testing for cervical cancer screening in First Nation women from Northwest Ontario, Canada: a pilot study. BMJ Open. 2011;1(1):e000030.

78. Cervarix [Product Monograph]. Ontario; 2019.

79. Gardasil [Product Monograph]. Quebec; 2015.

80. Gardasil 9 [Product Monograph]. Quebec; 2016.

81. Stanley M. HPV - immune response to infection and vaccination. Infect Agent Cancer. 2010;5:19.

82. Public Health Agency of Canada, National Advisory Committee on Immunization, Government of Canada. Updated recommendations on human papillomavirus (HPV) vaccines: 9-valent HPV vaccine and clarification of minimum intervals between doses in the HPV immunization schedule. Ottawa, Ontario: Public Health Agency of Canada; 2017.

83. Dobson SRM, McNeil S, Dionne M, Dawar M, Ogilvie G, Krajden M, et al. Immunogenicity of 2 Doses of HPV Vaccine in Younger Adolescents vs 3 Doses in Young Women: A Randomized Clinical Trial. JAMA. 2013;309(17):1793-802.

84. Sankaranarayanan R, Joshi S, Muwonge R, Esmy PO, Basu P, Prabhu P, et al. Can a single dose of human papillomavirus (HPV) vaccine prevent cervical cancer? Early findings from an Indian study. Vaccine. 2018;36(32):4783-91.

85. Markowitz LE, Dunne EF, Saraiya M, Chesson HW, Curtis CR, Gee J, et al. Human Papillomavirus Vaccination: Recommendations of the Advisory Committee on Immunization Practices (ACIP). Morbidity and Mortality Weekly Report: Recommendations and Reports. 2014;63(5):1-30.

86. Ismail S, Deeks S, National Advisory Committee on I. Summary of the National Advisory Committee on Immunization's Update on the recommended human papillomavirus (HPV) vaccine immunization schedule. Canada Communicable Disease Report. 2015;41(Suppl 3):11.

87. De Vincenzo R, Ricci C, Conte C, Scambia G. HPV vaccine cross-protection: Highlights on additional clinical benefit. Gynecologic Oncology. 2013;130(3):642-51.

88. Joura EA, Kjaer SK, Wheeler CM, Sigurdsson K, Iversen O-E, Hernandez-Avila M, et al. HPV antibody levels and clinical efficacy following administration of a prophylactic quadrivalent HPV vaccine. Vaccine. 2008;26(52):6844-51.

89. Villa LL, Ault KA, Giuliano AR, Costa RLR, Petta CA, Andrade RP, et al. Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. Vaccine. 2006;24(27):5571-83.

90. Nygård M, Saah A, Munk C, Tryggvadottir L, Enerly E, Hortlund M, et al. Evaluation of the Long-Term Anti-Human Papillomavirus 6 (HPV6), 11, 16, and 18 Immune Responses Generated by the Quadrivalent HPV Vaccine. Clinical and Vaccine Immunology. 2015;22(8):943-8.

91. Muñoz N, Manalastas Jr R, Pitisuttithum P, Tresukosol D, Monsonego J, Ault K, et al. Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24–45 years: a randomised, double-blind trial. The Lancet. 2009;373(9679):1949-57.

92. Villa LL, Perez G, Kjaer SK, Paavonen J, Lehtinen M, Munoz N, et al. Quadrivalent Vaccine against Human Papillomavirus to Prevent High-Grade Cervical Lesions. The New England Journal of Medicine. 2007;356(19):1915-27.

93. Villa LL, Costa RLR, Petta CA, Andrade RP, Ault KA, Giuliano AR, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncology. 2005;6(5):271-8.

94. Drolet MP, Bénard ÉB, Boily M-CP, Ali HP, Baandrup LMD, Bauer HMD, et al. Population-level impact and herd effects following human papillomavirus vaccination

programmes: a systematic review and meta-analysis. Lancet Infectious Diseases. 2015;15(5):565-80.

95. Racey CS, Albert A, Donken R, Smith L, Spinelli JJ, Pedersen H, et al. Cervical intraepithelial neoplasia rates in British Columbia women: A population level data linkage evaluation of the school-based HPV immunization program. The Journal of Infectious Diseases. 2019.

96. Geretti AM, Doyle T. Immunization for HIV-positive individuals. Current Opinion in Infectious Diseases. 2010;23(1):32-8.

97. Kroon FP, van Dissel JT. Antibody Response to Diphtheria, Tetanus, and Poliomyelitis Vaccines in Relation to the Number of $CD4^+$ T Lymphocytes in Adults Infected with Human Immunodeficiency Virus. Clinical Infectious Diseases. 1995;21(5):1197-203.

98. Irungu E, Mugo N, Ngure K, Njuguna R, Celum C, Farquhar C, et al. Immune Response to Hepatitis B Virus Vaccination Among HIV-1 Infected And Uninfected Adults in Kenya. The Journal of Infectious Diseases. 2013;207(3):402-10.

99. Neilsen GA, Bodsworth NJ, Watts N. Response to Hepatitis a Vaccination in Human Immunodeficiency Virus-Infected and -Uninfected Homosexual Men. The Journal of Infectious Diseases. 1997;176(4):1064-7.

100. Kernéis S, Launay O, Turbelin C, Batteux F, Hanslik T, Boëlle P-Y. Long-term immune responses to vaccination in HIV-infected patients: a systematic review and meta-analysis. Clinical Infectious Diseases. 2014;58(8):1130-9.

101. Giacomet V, Penagini F, Trabattoni D, Viganò A, Rainone V, Bernazzani G, et al. Safety and immunogenicity of a quadrivalent human papillomavirus vaccine in HIV-infected and HIV-negative adolescents and young adults. Vaccine. 2014;32(43):5657.

102. Kojic EM, Kang M, Cespedes MS, Umbleja T, Godfrey C, Allen RT, et al. Immunogenicity and safety of the quadrivalent human papillomavirus vaccine in HIV-1-infected women. Clinical Infectious Diseases. 2014;59(1):127-35.

103. Kahn JA, Xu J, Kapogiannis BG, Rudy B, Gonin R, Liu N, et al. Immunogenicity and safety of the human papillomavirus 6, 11, 16, 18 vaccine in HIV-infected young women. Clinical Infectious Diseases. 2013;57(5):735-44.

104. Toft L, Storgaard M, Müller M, Sehr P, Bonde J, Tolstrup M, et al. Comparison of the Immunogenicity and Reactogenicity of Cervarix and Gardasil Human Papillomavirus Vaccines in HIV-Infected Adults: A Randomized, Double-Blind Clinical Trial. The Journal of Infectious Diseases. 2013;209(8):1165-73.

105. Money DM, Moses E, Blitz S, Vandriel SM, Lipsky N, Walmsley SL, et al. HIV viral suppression results in higher antibody responses in HIV-positive women vaccinated with the quadrivalent human papillomavirus vaccine. Vaccine. 2016;34(40):4799-806.

106. WHO. Draft: Global Strategy Towards the Elimination of Cervical Cancer as a Public Health Problem. 2019.

107. Canadian Partnership Against Cancer. Canada acts to meet WHO Call to eliminate cervical cancer 2020 [Feburary 2020]. Available from: https://www.partnershipagainstcancer.ca/news-events/news/article/eliminate-cervical-cancer/.

108. Brisson M, Kim JJ, Canfell K, Drolet M, Gingras G, Burger EA, et al. Impact of HPV vaccination and cervical screening on cervical cancer elimination: a comparative modelling analysis in 78 low-income and lower-middle-income countries. Lancet. 2020.

109. Canfell K, Kim JJ, Brisson M, Keane A, Simms KT, Caruana M, et al. Mortality impact of achieving WHO cervical cancer elimination targets: a comparative modelling analysis in 78 low-income and lower-middle-income countries. Lancet. 2020.

110. Simms KT, Steinberg J, Caruana M, Smith MA, Lew J-B, Soerjomataram I, et al. Impact of scaled up human papillomavirus vaccination and cervical screening and the potential for global elimination of cervical cancer in 181 countries, 2020–99: a modelling study. The Lancet Oncology. 2019;20(3):394-407.

111. UN Women. Facts and figures: HIV and AIDS 2018 [Available from: https://www.unwomen.org/en/what-we-do/hiv-and-aids/facts-and-figures.

112. Gertig DM, Brotherton JML, Budd AC, Drennan K, Chappell G, Saville AM. Impact of a population-based HPV vaccination program on cervical abnormalities: a data linkage study. BMC Medicine. 2013;11(1):227-.

113. Ogilvie GS, Naus M, Money DM, Dobson SR, Miller D, Krajden M, et al. Reduction in cervical intraepithelial neoplasia in young women in British Columbia after introduction of the HPV vaccine: An ecological analysis. International Journal of Cancer. 2015;137(8):1931-7.

114. Blitz S, Baxter J, Raboud J, Walmsley S, Rachlis A, Smaill F, et al. Evaluation of HIV and highly active antiretroviral therapy on the natural history of human papillomavirus infection and cervical cytopathologic findings in HIV-positive and high-risk HIV-negative women. The Journal of Infectious Diseases. 2013;208(3):454.

115. World Health Organization. Comprehensive cervical cancer control: a guide to essential practice. Second ed. Geneva: World Health Organization; 2014.

116. UNAIDS. UNAIDS 2019 Estimates. 2019.

117. McClymont E, Ogilvie G, Albert A, Johnston A, Raboud J, Walmsley S, et al. Impact of quadrivalent HPV vaccine dose spacing on immunologic response in women living with HIV. Vaccine. 2020.

118. McClymont E, Lee M, Raboud J, Coutlée F, Walmsley S, Lipsky N, et al. The Efficacy of the Quadrivalent Human Papillomavirus Vaccine in Girls and Women Living With Human Immunodeficiency Virus. Clinical Infectious Diseases. 2019;68(5):788-94.

119. Brophy J, Bitnun A, Alimenti A, Lapointe N, Samson L, Read S, et al. Immunogenicity and safety of the quadrivalent human papillomavirus vaccine in girls living with HIV. Pediatric Infectious Disease Journal. 2018;37(6):595-7.

120. Small W, Bacon MA, Bajaj A, Chuang LT, Fisher BJ, Harkenrider MM, et al. Cervical cancer: A global health crisis. Cancer. 2017;123(13):2404-12.

121. Gallagher KE, LaMontagne DS, Watson-Jones D. Status of HPV vaccine introduction and barriers to country uptake. Vaccine. 2018;36(32):4761-7.

122. Abraham AG, D'Souza G, Jing YZ, Gange SJ, Sterling TR, Silverberg MJ, et al. Invasive Cervical Cancer Risk Among HIV-Infected Women: A North American Multicohort Collaboration Prospective Study. JAIDS. 2013;62(4):405-13.

123. Hankins C, Coutlee F, Lapointe N, Simard P, Tran T, Samson J, et al. Prevalence of risk factors associated with human papillomavirus infection in women living with HIV. Canadian Medical Association Journal. 1999;160(2):185-91.

124. Clifford GM, Goncalves MAG, Franceschi S, Grp HHS, Hpv, Group HIVS. Human papillomavirus types among women infected with HIV: a meta-analysis. AIDS. 2006;20(18):2337-44.

125. Coutlée F, Rouleau D, Petignat P, Ghattas G, Kornegay JR, Schlag P, et al. Enhanced Detection and Typing of Human Papillomavirus (HPV) DNA in Anogenital Samples with PGMY Primers and the Linear Array HPV Genotyping Test. Journal of Clinical Microbiology. 2006;44(6):1998-2006.

126. Tornesello ML, Buonaguro FM, Bethgiraldo E, Giraldo G. Human-Immunodeficiency-Virus Type-1 Tat Gene Enhances Human Papillomavirus Early Gene-Expression. Intervirology. 1993;36(2):57-64.

127. Massad LS, Xie X, Burk RD, D'Souza G, Darragh TM, Minkoff H, et al. Association of cervical precancer with human papillomavirus types other than 16 among HIV co-infected women. American Journal of Obstetrics and Gynecology. 2016;214(3):354.e1-.e6.

128. Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS. Persistent Human Papillomavirus Infection and Cervical Neoplasia: A Systematic Review and Meta-Analysis. American Journal of Epidemiology. 2008;168(2):123-37.

129. Arbyn M, Xu L, Simoens C, Martin-Hirsch PP. Prophylactic vaccination against human papillomaviruses to prevent cervical cancer and its precursors. The Cochrane database of systematic reviews. 2018;5:CD009069.

130. Markowitz LE, Dunne EF, Saraiya M, Lawson HW, Chesson H, Unger ER, et al. Quadrivalent Human Papillomavirus Vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP). Morbidity and Mortality Weekly Report: Recommendations and Reports. 2007;56(RR-2):1-23.

131. LaMontagne DS, Thiem VD, Huong VM, Tang Y, Neuzil KM. Immunogenicity of Quadrivalent HPV Vaccine Among Girls 11 to 13 Years of Age Vaccinated Using

Alternative Dosing Schedules: Results 29 to 32 Months After Third Dose. The Journal of Infectious Diseases. 2013;208(8):1325-34.

132. Russell K, Dunne EF, Kemper AR, Dolor RJ, Unger ER, Panicker G, et al. Antibody responses among adolescent females receiving the quadrivalent HPV vaccine series corresponding to standard or non-standard dosing intervals. Vaccine. 2015;33(16):1953-8.

133. Gilca V, Sauvageau C, Boulianne N, De Serres G, Crajden M, Ouakki M, et al. The effect of a booster dose of quadrivalent or bivalent HPV vaccine when administered to girls previously vaccinated with two doses of quadrivalent HPV vaccine. Human Vaccines & Immunotherapeutics. 2015;11(3):732-8.

134. Lazcano-Ponce E, Torres-Ibarra L, Cruz-Valdez A, Salmerón J, Barrientos-Gutiérrez T, Prado-Galbarro J, et al. Persistence of Immunity When Using Different Human Papillomavirus Vaccination Schedules and Booster-Dose Effects 5 Years After Primary Vaccination. The Journal of Infectious Diseases. 2019;219(1):41-9.

135. Sun X-W, Kuhn L, Ellerbrock TV, Chiasson MA, Bush TJ, Wright TC. Human Papillomavirus Infection in Women Infected with the Human Immunodeficiency Virus. The New England Journal of Medicine. 1997;337(19):1343-9.

136. Lowy DR, Herrero R, Hildesheim A, Participants in the INCIwoPEfPHPVVT. Primary endpoints for future prophylactic human papillomavirus vaccine trials: towards infection and immunobridging. The Lancet Oncology. 2015;16(5):e226-e33.

137. Castellsagué X, Muñoz N, Pitisuttithum P, Ferris D, Monsonego J, Ault K, et al. End-of-study safety, immunogenicity, and efficacy of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine in adult women 24-45 years of age. British Journal of Cancer. 2011;105(1):28-37.

138. Lord JM. The effect of ageing of the immune system on vaccination responses. Human vaccines & immunotherapeutics. 2013;9(6):1364-7.

139. WHO. Global Health Observatory Data. 2019.

140. J Ferlay IS, M Ervik, R Dikshit, S Eser, C Mathers, M Rebelo, DM Parkin, D Forman, F Bray. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancerbase No. 11. Lyon; 2013.

141. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global Cancer Statistics, 2012. CA: A Cancer Journal for Clinicians. 2015;65(2).

142. Koutsky LA, Harper DM. Chapter 13: Current findings from prophylactic HPV vaccine trials. Vaccine. 2006;24:S114-S21.

143. Wright TC, Ellerbrock TV, Chiasson MA, Devanter NV, Sun X-W. Cervical Intraepithelial Neoplasia in Women Infected With Human Immunodeficiency Virus: Prevalence, Risk Factors, and Validity of Papanicolaou Smears. Obstetrics & Gynecology. 1994;84(4, Part 1):591-7.

144. Clifford GM, de Vuyst H, Tenet V, Plummer M, Tully S, Franceschi S. Effect of HIV Infection on Human Papillomavirus Types Causing Invasive Cervical Cancer in Africa. Journal of Acquired Immune Deficiency Syndromes. 2016;73(3):332-9.

145. Massad LS, Silverberg MJ, Springer G, Minkoff H, Hessol N, Palefsky JM, et al. Effect of antiretroviral therapy on the incidence of genital warts and vulvar neoplasia among women with the human immunodeficiency virus. American Journal of Obstetrics and Gynecology. 2004;190(5):1241-8.

146. Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S, et al. Quadrivalent Vaccine against Human Papillomavirus to Prevent Anogenital Diseases. The New England Journal of Medicine. 2007;356(19):1928-43.

147. Luna J, Plata M, Gonzalez M, Correa A, Maldonado I, Nossa C, et al. Long-term follow-up observation of the safety, immunogenicity, and effectiveness of Gardasil™ in adult women. PloS One. 2013;8(12):e83431.

148. Giuliano AR, Palefsky JM, Goldstone S, Moreira ED, Penny ME, Aranda C, et al. Efficacy of quadrivalent HPV vaccine against HPV infection and disease in males. N Engl J Med. 2011;364.

149. Paavonen J, Naud P, Salmerón J, Wheeler CM, Chow SN, Apter D, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. The Lancet. 2009;374(9686):301-14.

150. Skinner SR, Szarewski A, Romanowski B, Garland SM, Lazcano-Ponce E, Salmerón J, et al. Efficacy, safety, and immunogenicity of the human papillomavirus 16/18 AS04-adjuvanted vaccine in women older than 25 years: 4-year interim follow-up of the phase 3, double-blind, randomised controlled VIVIANE study. Lancet. 2014;384(9961):2213-27.

151. Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, et al. A Controlled Trial of a Human Papillomavirus Type 16 Vaccine. The New England Journal of Medicine. 2002;347(21):1645-51.

152. Rowhani-Rahbar A, Mao C, Hughes JP, Alvarez FB, Bryan JT, Hawes SE, et al. Longer term efficacy of a prophylactic monovalent human papillomavirus type 16 vaccine. Vaccine. 2009;27(41):5612-9.

153. Wilkin TJ, Chen H, Cespedes MS, Leon-Cruz JT, Godfrey C, Chiao EY, et al. A randomized, placebo-controlled trial of the quadrivalent HPV vaccine in HIV-infected adults age 27 years or older: AIDS Clinical Trials Group protocol A5298. Clinical Infectious Diseases. 2018.

154. Ramanakumar AV, Goncalves O, Richardson H, Tellier P, Ferenczy A, Coutlée F, et al. Human papillomavirus (HPV) types 16, 18, 31, 45 DNA loads and HPV-16 integration in persistent and transient infections in young women. BMC Infectious Diseases. 2010;10(1):326-.

155. Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. Journal of the National Cancer Institute Monographs. 2003;2003(31):14-9.

156. Woodman CBJ, Collins S, Winter H, Bailey A, Ellis J, Prior P, et al. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. The Lancet. 2001;357(9271):1831-6.

157. Biggar RJ, Chaturvedi AK, Goedert JJ, Engels EA, HIV/AIDS Cancer Match Study. AIDS-Related Cancer and Severity of Immunosuppression in Persons With AIDS. Journal of the National Cancer Institute. 2007;99(12):962-72.

158. Franco EL, Burchell AN, Guenoun J, Tellier P-P, El-Zein M, Malagon T, et al. Estimating HPV DNA Deposition Between Sexual Partners Using HPV Concordance, Y Chromosome DNA Detection, and Self-reported Sexual Behaviors. Journal of Infectious Diseases. 2017;216(10):1210.

159. Kriek J-M, Jaumdally SZ, Masson L, Little F, Mbulawa Z, Gumbi PP, et al. Female genital tract inflammation, HIV co-infection and persistent mucosal Human Papillomavirus (HPV) infections. Virology. 2016;493:247-54.

160. Villa LL, Costa RLR, Petta CA, Andrade RP, Paavonen J, Iversen OE, et al. High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6 11 16 18 L1 virus-like particle vaccine through 5 years of follow-up. British Journal of Cancer. 2006;95(11):1459-66.

161. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. International Journal of Cancer. 2017;141(4):664-70.

162. Coutlée F, Ratnam S, Ramanakumar AV, Insinga RR, Bentley J, Escott N, et al. Distribution of human papillomavirus genotypes in cervical intraepithelial neoplasia and invasive cervical cancer in Canada. Journal of Medical Virology. 2011;83(6):1034-41.

163. Garland SM, Cornall AM, Brotherton JML, Wark JD, Malloy MJ, Tabrizi SN. Final analysis of a study assessing genital human papillomavirus genoprevalence in young Australian women, following eight years of a national vaccination program. Vaccine. 2018;36(23):3221.

164. Cespedes MS, Kang M, Kojic EM, Umbleja T, Godfrey C, Webster-Cyriaque JY, et al. Anogenital human papillomavirus virus DNA and sustained response to the quadrivalent HPV vaccine in women living with HIV-1. Papillomavirus Research. 2018;6:15-21.

165. McClymont E, Coutlée F, Lee M, Albert A, Raboud J, Walmsley S, et al. Brief Report: Persistence of Non-Vaccine Oncogenic HPV Genotypes in Quadrivalent HPV-

Vaccinated Women Living With HIV. Journal of Acquired Immune Deficiency Syndromes. 2020;83(3):230-4.

166. McClymont E, Lee M, Elwood C, Mitchell-Foster S, van Schalkwyk J, Ogilvie G, et al. Cervical Cancer Screening in Immunocompromised Women. Journal of Obstetrics and Gynaecology Canada. 2019;41(8):1177-80.