RESEARCH PROTOCOL

TITLE	Long Term Follow-up Study of CTN 236 – A Study of an HPV VLP Vaccine in a Cohort of HIV Positive Girls and Women
Abbreviated Title	Long Term Follow-up of HPV Vaccine in HIV (CTN 236)
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SUMMARY

Title

Long Term Follow-up Study of CTN 236 - A Study of an HPV VLP Vaccine in a Cohort of HIV Positive Girls and Women

Goal

We hypothesize that HPV antibody levels in HIV-positive girls and women will decline more rapidly and more significantly than in HIV-negative girls and women and that this decline will be determined by HIV parameters.

Study population

HIV-positive girls and women (greater than, or equal to, age 11) attending the HIV treatment clinic that are currently enrolled in CTN 236 study will be invited to participate in the 5 year long term follow-up study.

Objectives

Primary

To measure the antibody response to each genotype contained in the qHPV vaccine out to 96 months post-vaccination regimen.

Secondary

1) To determine the incidence rate and nature of 'breakthrough' HPV incident and persistent (2 sequential positive HPV DNA in > 6 months) infections of vaccine- non-vaccine-containing high-risk types;
2) To determine the incidence rate of cervical dysplasia (LSIL or greater) and/or vulvar and vaginal dysplasia associated HPV genotypes (both with and without vaccine- types); and
3) To determine the incidence rate of external genital warts.

Exploratory

1) To examine the relationship between HSV-2 serostatus and peak HPV antibody response as well as HPV incident and persistent infections; and
2) To relate vaginal microbiome profiles to HPV acquisition/persistence and cervical dysplasia.

Timeline

First Visit, First Subject (FVFS): 10 June 2015
Last Visit, Last Subject (LVLS): 30 March 2019

Study design

A longitudinal cohort follow up study of girls and women aged 11 years of age and older who participated in the study, "A Study of an HPV VLP Vaccine in a Cohort of HIV Positive Girls and Women (CTN 236)"

Inclusion Criteria

- Enrolled in CTN 236 study
- Able to give fully informed consent or assent

Exclusion Criteria

- Did not receive at least one vaccination via CTN 236, phase 1
- Cannot provide fully informed consent or assent

Duration of study for each subject

Up to 60 months
Subjects will participate in up to 3 possible study visits over a total of 5 years. Duration of time since vaccination as part of CTN 236 varies depending on when they were enrolled; therefore, to ensure all subjects are offered participation in the long term follow up, 6 possible visit times have been created.

Visit schedule

Main Study Procedures (no standard clinical or sub-study procedures)

	Informed Consent/ Assent	Health Questionnaire	Study Blood Work	Cytology and HPV DNA & vaginal swabs*	SAE and Con Med Assessment
Month 36 (Visit 8)	X	X	X	X	X
Month 48 (Visit 9)	X	X	X	X	X
Month 60 (Visit 10)	X	X	X	X	X
Month 72 (Visit 11)	X	X	X	X	X
Month 84 (Visit 12)	X	X	X	X	X
Month 96 (Visit 13)	X	X	X	X	X

* Those girls and women who are pre-menarchal or never sexually active will not have any pelvic examinations, clinical Pap smears, or study genital sampling until post-menarchal and sexually active.
Complete outline of all procedures found in table 7.3

Data collection

Template recruitment documents, consent and source documents will be provided by the Study Coordinating Centre. Source data will be transferred to paper case report forms and sent to the CIHR Canadian HIV Trials Network (CTN) where it will be entered into an electronic database.

Monitoring

This study is being conducted according to the following: International Committee on Harmonization (ICH) Guidance Document E6: Good Clinical Practice (GCP): Consolidated Guidelines. Site monitoring will be performed to ensure compliance with the above.

Data Safety Monitoring Board (DSMB)

The established CTN data safety monitoring committee with additional expertise in pediatrics and gyne-oncology will provide oversight and monitoring of the conduct of the study to ensure the safety of the subjects. Additionally, the DSMB will ensure the validity and integrity of the study data.

LIST OF ABBREVIATIONS

AE	Adverse event
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
BCCDC	British Columbia Centre for Disease Control
CPARG	Canadian Pediatrics AIDS Research Group
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in situ
CRF	Case report form
DAIDS	Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events
DNA	Deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
CTN	CIHR Canadian HIV Trials Network
GCP	Good clinical practices
HAART	Highly active antiretroviral therapy
hCG	Human Chorionic Gonadotropin
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
ICH	International Conference on Harmonization
IM	Intramuscular
NIH	National Institutes of Health
Pap	Papanicolaou cervical smear
PCR	Polymerase chain reaction
PID#	Primary identification number
PRN	When necessary
Q-HPV	Quadrivalent Human Papillomavirus Vaccine
REB	Research ethics board
RNA	Ribonucleic acid
SAE	Serious adverse event
SOP	Standard operating procedure
STI	Sexually transmitted infection
STM	Specimen transport media
VLP	Virus-like particles
WHRI	Women's Health Research Institute

1.0 Background

1. Epidemiology and Burden of Disease

Worldwide, cervical cancer is the fourth leading cause of cancer-related death in women with 528,000 new cases and 266,000 deaths yearly.[1]

Cervical cancer is the single greatest cause of death from cancer in women in sub-Saharan Africa, where HIV prevalence is exceptionally high particularly among young women.[1,2,3] In countries with wide access to quality Pap smear screening, the burden of disease has shifted to pre-invasive dysplastic lesions. In Canada, for example, there is at least 50 times more pre-invasive disease in women than there is cervical cancer, which still represents a substantial economic, social and psychological burden to women.[4,5] In spite of the pap screening program, there are over 350 deaths per year in Canada due to invasive cervical cancer (approximately 1 death every day)[6], often as a result of late diagnosis due to inadequate Pap smear screening, especially in vulnerable populations including immigrant and Aboriginal women.[7] This underlines a clear need to develop and evaluate preventative options.

The link between HPV and cervical cancer is well-established. HPV DNA is detected in 96.6% of cervical cancer tissue and the vast majority of cervical cancers can be attributed to HPV infection.[8] Based on both epidemiologic and phylogenetic data, the high-risk HPV types, for cervical cancer, in frequency order, are: 16, 18, 33, 45, 31, 58, 52 and 35, and ***probably high risk*** are: 51, 56, 39 and 59.[9] Overall, 70% of cervical cancer cases are caused by the two most common HPV types; 16 and 18 (high risk), and 90% of genital warts are caused by HPV 6 and 11 (low risk).[10]

The burden of disease in HPV infection outside the cervix is also important. HPV has been implicated in most non-cervical genital tract cancers, including penile and anal cancer, and in head and neck cancers. Cervical cancer incidence in a Canadian population is seen at 199.54 for 100,000 person/years (PY) in HIV positive women, compared to 47.5 per 100,000 PY in HIV negative women.[11] Fortunately, anal cancer has not been frequent in women, even women with HIV infection, with rates of 15.96 per 100,000 PY.[11] Research on the efficacy of this vaccine in preventing these cancers is also required.[12] As we did not conduct evaluations of baseline anal and oral HPV we are not in a position to conduct research into the efficacy of the HPV vaccine in this cohort to these types.

The affliction of disease from genital warts is also substantial. Data from the US suggests that genital warts are the most common sexually transmitted infection.[13] HPV 6 and 11 cause up to 90% of genital warts. In women, genital warts may develop anywhere in the lower genital tract including the cervix, with multiple sites being common. Clinically visible warts are present in approximately 1% of sexually active adults.[14] In persons with normal immune function, visible warts and evidence of viral replication are typically gone by approximately 18 months after initial

infection.[15] However, in HIV-positive individuals the lesions can be significantly larger and very problematic to treat, highlighting the benefits of HPV primary prevention in this population.[16,17] Of note, vaccination programs in HIV negative healthy populations are already showing decrease in genital warts,[18] but no data is available on this response in HIV positive vaccinated persons.

2. Clearance and persistence of HPV

The role of the host immune system in regression of HPV infection is not fully understood but in regressing genital warts an infiltration can be detected of CD4 cells, CD8 cells and macrophages that likely represents a T-cell response to specific early proteins of HPV (E2 and E6).[19] In HIV-negative women, most of these infections (80%) appear to 'clear'; that is, HPV DNA can no longer be detected on the mucosal or epithelial surface. The time required for this 'clearance' appears to vary with different HPV genotypes; ranging from 5-6 months for low risk types to 8-14 months for high-risk types.[20,21,22] It is unclear whether the virus is completely eliminated, or if it remains latent in basal cells and could reactivate under immune permissive conditions. Risk of carcinogenesis appears to be directly related to the persistence of oncogenic (high risk) virus. The definition of transient or persistent infection has been defined in most studies as the presence of HPV DNA by polymerase chain reaction (PCR) at two consecutive assessments 6 months apart.[23]

3. Natural HPV Immunology

Many nuances of HPV immunology are poorly understood, in particular the ability of the virus to evade the immune system. Natural immunity is generally not effective in protecting against re-infection with the same genotype.[24] Our current understanding is that the host's immune response to natural infection initially involves a local innate response, which then triggers circulating humoral (antibody) and cell mediated responses (e.g. CD4 and CD8 cells). The cell-mediated immune response is required for HPV containment and lesion regression.[13,25] Persons with cell-mediated immune dysfunction, such as HIV-positive individuals, have higher rates of HPV infection. **HIV-positive women have much higher rates of cervical dysplasia and cancer than HIV-negative women and progress more rapidly from low grade to high grade lesions and to invasive cancer.**[26,27] Of note, our recent study of cervical cancer in HIV-positive women in British Columbia has determined that HIV-positive women have 4.2 fold (2.72-6.20) higher rate of HPV associated female genital cancer than the general population.[11] The baseline rate of low-grade cervical dysplasia is approximately 13% in HIV-positive women compared to 4% in HIV-negative women.[27,28] The problem is exacerbated in HIV endemic areas such as sub-Saharan Africa where Pap smear screening programs are largely lacking.[29]

4. HPV/ HIV interactions

The T cell immune dysfunction caused by HIV infection offers a unique opportunity to study and better understand the interrelationship between the neutralizing antibody protection of the humoral immune system triggered by the vaccine, and HPV elimination that requires adequate T cell function. **The most valuable knowledge gained in this area will be a definition of an immune correlate of protection, i.e. a level of circulating antibody above which the majority of persons will be protected from acquisition of the infection. This new knowledge will be highly relevant to vaccine programming in Canada and globally.**

The interactions between HIV and HPV are not fully understood. HIV-mediated immune suppression appears to facilitate HPV persistence and its oncogenic potential. In part this appears to be due to direct enhancement of HPV integration in the presence of HIV.[30] The effect of HPV on HIV replication and viral load in the circulation and in the genital tract is not well characterized. Of note, HPV studies have typically involved interval evaluations of HPV shedding which has led to a poor understanding of the longitudinal process of infection, persistence, clearance, and reinfection or reactivation.[23] In some studies to date, combination antiretroviral therapy (cART), presumably through immune function reconstitution, results in higher rates of clearance compared to those not on cART.[31,32,33] However, in other studies, cART and CD4 counts have not been well correlated with clearance and in fact HIV suppression and inversely HIV replication (higher viral load) has been associated independently.[34] Some of the difficulty has been the prior study designs. Data from our study group prior to the availability of HPV vaccine showed that there was a differential response to immune suppression and infection with HPV 16 in that perhaps HPV 16 is such a potent oncogenic genotype that it can cause disease regardless of immune function.[24,31]

5. HPV prevalence is high in HIV-positive cohort studies

Our studies and others have shown that HPV is highly prevalent in HIV positive populations. Even in a post HAART era, clearance is still diminished compared with HIV negative populations and this underscores the need to better understand the role of HIV replication and HPV infection and the HPV immune response. Data from the Canadian Women's HIV study group found high risk HPV genotypes, as detected by genital tract sampling in HIV-infected women, in the following order of frequency: 52, 84, 16 and 83.[35] In this cohort (n=198, samples=437), 62.1% of women were HPV positive at least once for one or more of the 27 HPV types tested, with 7.6% positive for 6/11, 15.7% positive for 16/18, and 1.0% positive for 6/11 & 16/18. In an international study, 77% of HIV-positive women had HPV detected at least once (incident infection). HPV 16 was the most frequent genotype, with mixed infection seen in 26%.[36] Persistent infection was seen in 41.5% of women but there was no relationship between HPV infection status and CD4 cell count or HIV viral

load. This compares to a 2.6% HPV 16 persistence seen in the HIV-negative women in the placebo arm of the large HPV 16/18 trial.[37] Furthermore, an improved response to treatment for cervical dysplasia was observed for women on highly active antiretroviral therapy (HAART).[38] Similar data were seen in other cohorts including a South African study demonstrating rapid increase in HPV infection after HIV seroconversion[39] and in a US cohort with relatively high HPV prevalence and higher rates of cervical dysplasia in women with lower CD4 cell counts.[40] Some studies have shown a similar 16, 18 genotype dominance in HIV-positive women compared to HIV-negative women in causing cervical dysplasia and cancer.[41,42,43,44] However in other studies multiple type infections and non 16/18 oncogenic HPV were associated with persistence, cervical dysplasia and cancer, highlighting the importance of cross-protection, and/or multivalent vaccines in HIV positive populations.[45,46,47]

6. HPV, other STI's and the vaginal microbiome

Elucidating the role of HSV-2 in contributing to HPV persistent infection and oncogenic progression is important because HSV-2 co-infection is common, with an estimated seroprevalence of 55% in Canadian HIV-infected populations.[48] Safe and inexpensive antiviral drugs can be used both for direct treatment but also for treatment as prevention by suppressing HSV viral shedding in an infected person and substantially decreasing risk of transmission to an uninfected person.[49,50] Existing data on the role of HSV-2 in cervical dysplasia are unclear. While several studies have observed an association between HSV-2 co-infection (defined either serologically or by the presence of HSV-2 DNA in cervical samples) and cervical cancer, this relationship has sometimes[51,52,53,54] but not always[55,56] persisted after adjustment for HPV DNA. However, most studies were limited by their use of HSV-2 serology assays with poor specificity (e.g. based on whole virus or crude lysate[57]), and to our knowledge there are no prior studies in the setting of HIV infection. The rich clinical and microbiologic data within our HPV vaccine in HIV positive girls and women study provides a unique opportunity to evaluate the role of herpes simplex virus type 2 (HSV-2) as a cofactor in progression to cervical dysplasia among HIV-infected women.

There has been increasing evidence of the interrelationship between the composition of the endogenous lower genital tract microbiota and the risk of acquisition and/or transmission of genital pathogens, including HIV and HPV.[58,59,60,61,62] These findings suggest there are relationships between microorganisms in a given niche, and provide evidence to support the hypothesis that a healthy vaginal microbiome may be protective against pathogens. Dysbiosis of the vaginal microbiome is associated with increased susceptibility to human papillomavirus (HPV) infection, and may also influence progression of HPV to cervical cancer.[63,64] Although we understand that HPV infection is a prerequisite for the development of cancer of the uterine cervix, we are also beginning to understand the ability of other infections or perturbations of the vaginal ecology to affect acquisition of HPV, persistence of HPV, and the ability of this virus to cause cervical dysplasia and cancer. The complexity of this

interplay is intricate and only understood in a very limited fashion. A microbial ecology approach is required to fully understand these phenomena. Our investigator team, which includes members funded through the CIHR Canadian Microbiome Initiative to study the vaginal microbiome utilizing a metagenomic approach, is well equipped to explore these issues. This collective expertise will permit the profiling of the bacteriome associated with oncogenic HPV persistence and/or integration into the host genome, and will enable future personal profiling of cervical cancer risk based on microbiome profiles.

7. HPV Vaccines

The two currently licensed vaccines designed to prevent HPV infection, have been tested in large clinical trials. There is a bivalent vaccine against HPV 16 and 18 (GlaxoSmithKline, GSK) and a quadrivalent vaccine against HPV 6, 11, 16 and 18 (Merck). In addition, a 9-valent vaccine is currently being investigated in large scale clinical trials, with positive safety, immunogenicity and efficacy data under review by the FDA. These vaccines are produced as virus-like particles (VLP's) based on the LI capsid protein gene of the virus inserted in insect or yeast vectors. These proteins self-assemble into protein shells with identical configuration to the native virus, but without DNA, and so are deemed subunit, protein vaccines. Safety, efficacy, and immunogenicity data on these vaccines in HIV-negative populations of young women are based on clinical trials conducted by GSK and Merck, respectively, with some follow up post-licensure trials now in progress. Both have shown remarkable safety profiles in otherwise healthy young HIV-negative women, with limited local injection site reactions as the primary side-effect and no evidence for differences in serious adverse events between vaccine and placebo arms.[65]

Immunogenicity to the quadrivalent vaccine has been well studied in HIV-negative populations, showing that anti-HPV 6, 11, 16 and 18 geometric mean titers (GMT) were highest at month 7 (1 month following the final dose 3). Anti-HPV GMT's declined thereafter but reached a plateau at month 24 and remained stable through 5 years.[66,67,68] In the original immunogenicity study by Villa et al.,[67] 100% of the subjects who were antibody negative to the relevant HPV type at baseline had a detectable antibody response at month 7 to HPV 6, 11, 16 and 18 respectively. These levels were 10-to 10^4-fold higher than natural antibody levels in placebo recipients who were vaccine type positive at baseline.

8. HPV vaccine implementation

Currently, Canada has established a population level HPV vaccination strategy through school-based publicly-funded programming. As yet, the impact of this is limited by uptake and by the relatively young age of the public based program. Individuals who are not eligible in the public program and who have the means are able to pay for the vaccine, but vulnerable populations who cannot afford the vaccine lack access and continue to acquire HPV infection unnecessarily. It is vital to understand how best to protect our most vulnerable persons from HPV infection

and the risk of invasive cervical cancer. The implications of the vaccine programme for Pap smear screening is yet to be fully understood and therefore current screening schedules have not been adjusted. It is hoped that the vaccine will be particularly useful in preventing disease in women who have difficulty accessing Pap smear screening, e.g. Aboriginal women and Asian immigrant women who have shown low access to and uptake of Pap smear screening.[69,70,71,72,73]

9. Global HPV vaccine implementation plans

Globally 266,000 women die every year, and without significant changes, this is estimated to be 416,000 by 2035. In resource-limited areas such as Africa, with little or no access to Pap smear screening, cervical cancer not only kills women, but its incidence is greatly exacerbated by the high prevalence of HIV infection. Cervical cancer incidence in South Africa is 174.8 per 100,000. In India, it is even higher at 186.5 per 100,000.[74] Safe and effective HPV vaccines would be of immense value in countries with such high rates of cervical cancer, especially in the absence of widely implemented screening programs. The World Health Organization (WHO) has demonstrated the capability of implementing large-scale vaccine programs. For example in 2003, global coverage of three doses of the diphtheria-tetanus-pertussis combination vaccine reached 78%.[75] Even complicated, comprehensive antiretroviral therapy rollout has been impressive with UNAIDS estimating that almost 10 million people in resource-constrained countries were on antiretroviral therapy at the end of 2012.[76] The Global Alliance for Vaccine and Immunisation (GAVI) is negotiating affordable three-tiered pricing for countrywide roll-out now with a number of low-income countries. The rational implementation of HPV vaccination programs in these areas is dependent on a sound understanding of the impact, safety, and efficacy of vaccination in HIV-positive persons who may be at varying stages of immune dysfunction.[77] As of February 4 2014, 10 additional countries were added as recipients of HPV vaccine rollout (to a total of 21 countries). This is to be primarily implemented using a 3 dose schedule but some pilot projects have opted for a 2 dose schedule, based on Canadian data from some of our investigator group. Although, we are confident in healthy Canadian HIV-negative girls, 2 doses of the vaccine provides equivalent antibody levels to 16-26 year old women who are well protected with these levels, this dosing regimen has not been tested in HIV positive populations. This greatly increases the urgency to determine the immunogenicity and efficacy for areas with high rates of HIV prevalence.

10. HPV vaccine in HIV positive studies

Our study group was one of the first groups globally to study one of these vaccines in HIV-positive, immune compromised populations. As the qHPV vaccine GARDASIL™ (Merck), which contains subtypes 6 and 11 (responsible for 90% of genital warts) and subtypes 16 and 18 (responsible for 70% of cervical cancer) was the first to be licensed and available in Canada for post-licensure studies, we engaged with Merck to permit an initial study of the safety and immunogenicity of this vaccine in a cohort of HIV-positive girls and women. Data from this cohort has

provided valuable information about the health of Canadian women and girls living with HIV. At the initial screening visit more than 80% of subjects were negative to HPV genotypes 6, 11, 16 and 18, found in the qHPV vaccine. This was a higher seronegative rate than seen in a US based study in which only 55.6% were HPV 16 negative and 73.7% HPV 18 negative at baseline, despite being a younger cohort overall (N=99, mean age 21.4 years).[78] Preliminary data outlined that in our study, 17 % of HIV-positive women had low-grade squamous intraepithelial lesions (LSIL) or greater at screening and 29% had at least one strain of oncogenic HPV present. It was observed that greater numbers of oncogenic HPV types were present in those women with cervical dysplasia.[79]

Antibody response to HPV types 6, 11, 16 and 18 has been collected and analyzed on over 1500 serology samples for months 0, 7, 12, 18 and 24. Seroconversion rates at month 7 were approximately 93-98% in this HIV-positive population, much higher than anticipated. However, peak Geometric Mean Titres (GMT) were lower than in HIV negative populations. In addition this was associated with a significant decline in antibody levels by month 24, particularly to HPV18. Similar to our findings, Kahn et al., also showed that level of antibody was lower in individuals not on cART in their open label 16-23 year old study of 99 women given the q HPV vaccine.[80] Of note, we also observed statistically significantly higher levels of incident and persistent infections of vaccine containing types in a short term follow up period. This preliminary data outlines the importance of following this established cohort for long-term immunogenicity. When directly comparing the school based vaccine target age of 9-13 years, the HIV positive girls had a statistically significantly lower peak GMT than age matched HIV comparators.[81,82] Similar studies conducted by other investigator groups have preliminary data available as well. Weinberg et al, showed that in HIV infected 7-12 year old children, there was a good seroconversion rate at > 94% but noted that the level of antibody to HPV18 was less than for the other genotypes. They also added a fourth dose of the vaccine which demonstrated an appropriate anamnestic response suggesting that should a booster be deemed necessary it should function to appropriately enhance the antibody response.[83] A South African based study of the bivalent vaccine demonstrated safety and 100% seroconversion to the vaccine containing serotypes regardless of CD4, HIV viral load and HIV clinical stage of disease.[84]

Our study will ensure careful assessment and scrutiny of the health outcomes in HIV-positive girls and women who were able to access the HPV quadrivalent vaccine (GARDASIL™) in our previous study. We will be able to determine the value of this vaccine for sub-groups of girls and women with CD4 T cell depletion and variable HAART uptake. Most studies published to date on HPV vaccines have been, by necessity, supported by the vaccine manufacturing companies, and have excluded HIV-infected subjects. This study remains one of the few investigator-driven, peer-reviewed, independently funded vaccine studies, allowing for open scrutiny of vaccine efficacy and safety in a typically understudied population.

2.0 HYPOTHESIS

It is hypothesized that HPV antibody levels in HIV-positive girls and women will decline more rapidly and more significantly than in HIV-negative girls and women and this decline will be determined by HIV parameters.

3.0 OBJECTIVES

The overall goal of the study is to determine the immunogenicity and efficacy of the HPV vaccine out to 4 years in an established cohort of HIV-positive girls and women in Canada.

1. **Primary**

   To measure the antibody response to each genotype contained in the qHPV vaccine out to 96 months post vaccination regimen.

2. **Secondary**

   1) To determine the incidence rate and nature of 'breakthrough' HPV incident and persistent (2 sequential positive HPV DNA in > 6 months) infections of vaccine- non-vaccine-containing high-risk types;
   2) To determine the incidence rate of cervical dysplasia (LSIL or greater) and/or vulvar and vaginal dysplasia associated HPV genotypes (both with and without vaccine- types); and
   3) To determine the incidence rate of external genital warts.

3. **Exploratory**

   1) To examine the relationship between HSV-2 serostatus and peak HPV antibody response as well as HPV incident and persistent infections;
   2) To relate vaginal microbiome profiles to HPV acquisition/persistence and cervical dysplasia.

4.0 STUDY DESIGN & METHODS

1. **Design**

   This prospective longitudinal cohort study will evaluate the effectiveness of a quadrivalent HPV VLP vaccine in an HIV-positive cohort of girls and women in multiple centres across Canada.

2. **Rationale**

   This study presents a unique opportunity to understand the long term impact of HPV vaccination in immune compromised women. Our prior study demonstrated that although women and girls living with HIV in Canada have complicated lives and have a high burden of socioeconomic disadvantages, they are very interested and committed to participating in research. This is clearly demonstrated by the degree of engagement of positive women in the National Consensus Statement on Women, Trans People and Girls and HIV Research in Canada. Our study sites across the country have been highly effective at engaging with this challenging population

through their long-standing, dedicated clinics. We have a cohort of over 400 girls and women who continue to live with their HIV disease and will require ongoing specialized HIV care for the rest of their lives. As they are just completing the first protocol out to 24 months and are for the most part still attending for care, with 94% remaining engaged with our study groups. We are confident that we can effectively re-engage them in our follow-up study. It is vital that this study be launched now while most participants are at 24-36 months from vaccine course, and key follow up timed sampling can ascertain the trajectory of antibody responses as well as the course of HPV disease.

Parallel studies in HIV-negative women, some being conducted by members of our investigator group, in Canadian cohorts, are ideal for comparison purposes. For example, there are over 1,000 girls enrolled in Quest, the long term follow up of the 2 dose/3 dose study of the qHPV vaccine in healthy volunteers in Canada (Dobson/Ogilvie). This permits patient level data to be compared directly with inclusion of known confounders in the analysis. We have support from Merck (see appended letter of support) for the competitive Luminex immunoassay (cLIA) assay to ensure consistency and comparability to both our prior study antibody titres and to the published literature on qHPV antibody responses. To date, all qHPV vaccine studies in the published literature, including those conducted by our investigator group, have had the antibody assays conducted through Merck laboratories. This provides an extraordinary ability to conduct robust comparisons of immune responses across study populations who have been given the qHPV vaccine.

5.0 STUDY COHORT

1. Population

Four hundred and seven (407) HIV-positive girls and women (greater than, or equal to, age 11) attending the HIV treatment clinics in each of the 13 sites across Canada and who have enrolled in the original study "A Study of an HPV VLP Vaccine in a Cohort of HIV Positive Girls and Women (CTN 236)" will be offered participation in the study.

Inclusion Criteria

- Enrolled in CTN 236 study
- Able to give fully informed consent or assent

Exclusion Criteria

- Did not receive at least one vaccination via CTN 236, phase 1
- Cannot provide fully informed consent or assent

2. Delays and contraindications to subsequent study procedures

5.2.1 Indications for a delay in genital sampling

- Pre-menarchal and not sexually active – no genital sampling until post-menarchal and sexually active or at the discretion of the investigator
- Subjects must not be menstruating at the time of a genital sampling. A study visit may need to be delayed until menses has stopped or the subject may have all other study procedures performed and the subject will have to be brought back to the clinic for genital sampling.

6.0 STUDY CONDUCT

1. Adherence to Industry Guideline and Regulations

This trial is to be conducted in accordance with the protocol and ICH GCP.

All study staff should be familiar with the following document**:**
ICH GCP: http://www.ich.org/cache/compo/276-254-1.html

All investigators and their investigative sites are obligated to comply with investigator responsibilities, monitoring, and archiving data, audits, confidentiality, and publication requirements.

The Study Coordinating Centre shall also accept the responsibilities of the Sponsor as outlined in ICH GCP.

2. Ethical conduct / research ethics board (REB)

Each site will be required to utilize a REB that conforms to section 3 of the GCPs: 3.1 Responsibilities, 3.2 Composition, Functions and Operations, 3.3 Procedures, 3.4 Records.

Prior to the conduct of this trial, the protocol, consent form, and all related documents must be submitted to the REB at each site. Approval for this study must be granted before the trial can be started at that site.

The Principal Investigator at each site will forward a copy of the REB approval to the Study Coordinating Centre before the first subject is enrolled in the study at that site. All amendments to the protocol that affect the safety and/or welfare of the study participants shall be submitted to the site REB and approved prior to implementation.

All study staff should be familiar with the Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans:
http://www.pre.ethics.gc.ca/english/policystatement/policystatement.cfm

3. Informed Consent/ Assent

Typically, each subject and parent/legally authorized representative (if applicable) must participate in the informed consent process and sign and date the informed consent form before any procedures specific to this protocol are performed on that subject.

A modified recruitment method may be utilized if clinical care flow for a patient would be excessively interrupted by research. To ensure clinical history and pelvic examination occur in a timely manner, a HPV in HIV researcher may not always have the opportunity to speak with a potential study participant prior to a pelvic examination. In these circumstances prior to the routine pelvic exam, the attending clinician will request verbal permission to collect two additional vaginal swab samples and a cytology sample during the exam for the purposes of this study. Ability to verbally consent, in these instances, will better respect both clinician and patient time and reduce participation burden by allowing for study sample collection to align with clinical sample collection.

After the exam, the HPV in HIV researcher will immediately follow up and meet with the patient to go over the full consent form, and answer any questions or concerns prior to their signing the informed consent document. If written informed consent is not provided, samples will be discarded.

Consenting *a posteriori* will confer benefits to the participating women as their clinical care flow will not be interrupted by research. Also, by obtaining verbal consent at the initial pelvic exam to obtain samples, we will minimize the discomfort, stress and inconvenience experienced by women that could otherwise be subjected to a second pelvic exam.

In the absence of a site SOP for informed consent, the site will write a note to file explaining the procedures followed while obtaining informed consent/assent.

At each subsequent study visit, subjects and parent/legally authorized representative (if applicable) will be asked if they wish to continue in the study and give their ongoing consent/assent to participate. This will be documented in the source documentation.

In accordance with the ICH Harmonized Tripartite Guidelines for GCP for those subjects who can be enrolled in the trial with the consent of the subject's legally acceptable representative (e.g. minors), the subject should be informed about the trial to the extent compatible with the subject's understanding and, if capable, the subject should sign and personally date a written informed assent. It is required that the assent be signed by each subject, if capable, in addition to the informed consent that is to be signed by her legal representative.

A young subject who originally signed an assent form who becomes menarchal and sexually active will be required to sign a consent form that includes the study genital sampling prior to performing any of these procedures.

Study consent forms and assent forms and above outlined use of verbal consent must be approved by a REB at the particular study site.

An informed consent form template and assent form template are included in the protocol appendices and may be amended as per site and REB requirements. REB approved consent and assent forms for each site are to be sent to the Study Coordinating Centre and updated as necessary.

4. Source Documentation

Study source documentation should be kept for each subject at the study site. Source data is raw data such as original laboratory records, documentation, documentation of original observations and activities. Source data is the first time and place an observation is documented, for example the documentation of a blood pressure.

Study source documentation should be captured separately in the subject study chart; however, when the original source data is such that it cannot be placed with the subject source document chart, for example, the source data is contained in the hospital patient chart, sites may take a photocopy of the document and indicate on the photocopy where the data was first documented.

When source data is in electronic form, for example, laboratory results, a printout of the electronic data is to be printed and placed in the subjects source document chart.

A source document template will be provided to sites by the study coordinating centre. However, sites are not required to use the template if other approved methods of capturing source data are preferred. Alternate methods of capturing source data must be approved by the Study Coordinating Centre.

5. Monitoring

A representative of the Study Coordinating Centre will visit investigative sites during the first months of enrollment to ensure that all procedures are consistent with the protocol and that appropriate Standard Operating Procedures (SOPs) and essential study documentation are on file.

The sites will be monitored to ensure that the trial is being conducted in accordance with the protocol and ICH GCP.

Consent documentation will be reviewed at 100%. Source documentation/CRF reconciliation will occur at 20% at each site. Monitoring at each site will be based on enrollment at that site. A close-out of the study will occur after the Last Patient Last Visit has been conducted at each site.

Enrollment and study progress reports will be requested of the study sites at a frequency to be determined by enrollment rates.

7.0 STUDY PROTOCOL

1. Participant identification number (PID#) assignment

For this long term follow-up study of CTN 236, subjects will not be assigned new study numbers and will remain with the study number assigned as part of CTN 236.

7.1.1 Table of Site Numbers

Site Number	Study Site
01	Oak Tree Clinic - Children and Women's Health Centre of BC, Vancouver
05	Toronto General Hospital, Toronto
06	Maple Leaf Medical Clinic, Toronto
07	St. Michael's Hospital, Toronto
09	Hospital for Sick Children, Toronto
10	McMaster University Hospital, Hamilton
11	Kingston General, Kingston
12	Children's Hospital of Eastern Ontario, Ottawa
13	McGill University Health Centre, Montreal
14	CHU Sainte Justine, Montreal
15	Centre Hospitalier de l'Université Laval, Québec City
17	HIV Care Program, Windsor
21	St Paul's Hospital, Vancouver

2. Considerations for special groups

7.2.1 Females aged 11-18 who are pre-menarchal and not sexually active

Adolescents and young women will be offered enrollment in the setting of established and experienced pediatric clinics. There are young girls with HIV infection, primarily perinatally acquired, who may be unaware of their diagnosis and the actual terminology of HIV. This is standard practice for care of children up to age 12 with a complex diagnosis with profound social stigmatization issues.

Those that are aware of the HIV terminology will receive the information and consents with HIV specific information. The term "immunocompromised" has been used in place of HIV in documents and

templates that may be viewed by these young women (e.g. consent forms). Each site may use the standard terminology used at their site.

Those girls and women who are pre-menarchal and not sexually active will not have the same degree of disease endpoint evaluations (i.e. will not have any pelvic examinations or Pap smears until post menarchal and sexually active or at the discretion of the site investigator).

A young subject who originally signed an assent form who becomes menarchal and sexually active will be required to sign a consent form that includes the study genital sampling prior to performing any of these procedures.

7.2.2 Changes to study procedures in pregnancy

In the event of pregnancy Pap testing and genital study sampling will continue; however, an Ayre spatula will be used to collect cervical samples in place of the cytobroom until 3 months post-pregnancy.

3. Summary table of study visits

	Visit Window	Ongoing Consent/Assent	Incl/Excl or Contraindication Criteria	Clinical Laboratory Investigations	Study Blood-Work (serology)	Pelvic Exam& Vaginal Sample Collection *	Study Cervical Cytology and HPV DNA *	SAE/Med Hx and Con Med/Exposure Update
Month 36 (Visit 8)	First Dose + 36 months (+/- 2 months)	X	X	X	X	X	X	X
Month 48 (Visit 9)	First Dose + 48 months (+/- 2 months)	X	X	X	X	X	X	X
Month 60 (Visit 10)	First Dose + 60 months (+/- 2 months)	X	X	X	X	X	X	X
Month 72 (Visit 11)	First Dose + 72 months (+/- 2 months)	X	X	X	X	X	X	X
Month 84 (Visit 12)	First Dose + 84 months (+/- 2 months)	X	X	X	X	X	X	X

Month 96 (Visit 13)	First Dose + 96 months (+/- 2 months)	X	X	X	X	X	X	X

* Those girls and women who are pre-menarchal and not sexually active will not have any pelvic examinations or Pap smears until post-menarchal and sexually active or at discretion of the site investigator.

Due to variation in duration of time since first dose of vaccine, some subjects are out of window for Month 36 and Month 48, e.g. it has been more than 48 months since first dose of vaccine was received. Therefore, we have extended the study visit timeline up to and including Month 96 post-vaccine, also designated Visit 13. Visits will occur annually until project grant is completed (March 31, 2019).

4. Study Visits

7.4.1 Ordering of Study Procedures

At all visits - Study blood for serum antibody and cervical cytology/HPV DNA test and vaginal samples are to be done within a 24 hour period. Any deviations to the above exceptions must be documented in the source document and reported to the Study Coordinating Centre.

One time point - Lower Urinary Tract Symptoms Survey
This survey is a one-time cross sectional measure of lower urinary tract symptoms in participants above age 18. Questionnaires will be completed varying months post-vaccination, in line with varying enrollment dates in the precursor study.

7.4.2 Study Visit Window

Interval	Length of interval (months)	Recommended interval between scheduled visits (months)
First dose to Month 36/Visit 8	(36 ± 2)	12
First dose to Month 48/Visit 9	(48 ± 2)	12
First dose to Month 60/Visit 10	(60 ± 2)	12
First dose to Month 72/Visit 11	(72 ± 2)	12
First dose to Month 84/Visit 12	(84 ± 2)	12
First dose to Month 96/Visit 13	(96 ± 2)	12

7.4.3 Outline of study visits

MOTNH 36/VISIT 8 (36 months from first vaccine):

- Assess ongoing consent/assent
- Health history update, Pap testing/treatment outside study, and any new/resolved health issues
- Update concomitant medication and exposure usage

- Review study blood work/genital sampling delay and contraindication criteria
- Height and weight
- Clinical laboratory investigations including resistance testing
- Study serology
- Assess menarchal and sexually active status

Post menarchal and sexually active girls and women and/or at the discretion of the site investigator ONLY:

- Pelvic examination, including
  - external genital examination (including genital warts assessment)
  - inspection of vagina and cervix
  - additional assessments as per local practice and/or clinically indicated (e.g. sexually transmitted infection screening samples, bacterial vaginosis, clinical Pap smear, etc.)
- Liquid based study cervical cytology and HPV DNA sample
- Vaginal microbiota swabs

MONTH 48/VISIT 9 (48 months from first vaccine):

- Assess ongoing consent/assent
- Health history update, Pap testing/treatment outside study, and any new/resolved health issues
- Update concomitant medication and exposure usage
- Review study blood work/genital sampling delay and contraindication criteria
- Height and weight
- Clinical laboratory investigations including resistance testing
- Study serology
- Assess menarchal and sexually active status

Post menarchal and sexually active girls and women and/or at the discretion of the site investigator ONLY:

- Pelvic examination, including
  - external genital examination (including genital warts assessment)
  - inspection of vagina and cervix
  - additional assessments as per local practice and/or clinically indicated (e.g. sexually transmitted infection screening samples, bacterial vaginosis, clinical Pap smear, etc.)
- Liquid based study cervical cytology and HPV DNA sample
- Vaginal microbiota swabs

MONTH 60/VISIT 10 (60 months from first vaccine):

- Assess ongoing consent/assent
- Health history update, Pap testing/treatment outside study, and any new/resolved health issues
- Update concomitant medication and exposure usage
- Review study blood work/genital sampling delay and contraindication criteria

- Height and weight
- Clinical laboratory investigations including resistance testing
- Study serology
- Assess menarchal and sexually active status

Post menarchal and sexually active girls and women and/or at the discretion of the site investigator ONLY:

- Pelvic examination, including
  - external genital examination (including genital warts assessment)
  - inspection of vagina and cervix
  - additional assessments as per local practice and/or clinically indicated (e.g. sexually transmitted infection screening samples, bacterial vaginosis, clinical Pap smear, etc.)
- Liquid based study cervical cytology and HPV DNA sample
- Vaginal microbiota swabs

MONTH 72/VISIT 11 (72 months from first vaccine):

- Assess ongoing consent/assent
- Health history update, Pap testing/treatment outside study, and any new/resolved health issues
- Update concomitant medication and exposure usage
- Review study blood work/genital sampling delay and contraindication criteria
- Height and weight
- Clinical laboratory investigations including resistance testing
- Study serology
- Assess menarchal and sexually active status

Post menarchal and sexually active girls and women and/or at the discretion of the site investigator ONLY:

- Pelvic examination, including
  - external genital examination (including genital warts assessment)
  - inspection of vagina and cervix
  - additional assessments as per local practice and/or clinically indicated (e.g. sexually transmitted infection screening samples, bacterial vaginosis, clinical Pap smear, etc.)
- Liquid based study cervical cytology and HPV DNA sample
- Vaginal microbiota swabs

MONTH 84/VISIT 12 (84 months from first vaccine):

- Assess ongoing consent/assent
- Health history update, Pap testing/treatment outside study, and any new/resolved health issues
- Update concomitant medication and exposure usage
- Review study blood work/genital sampling delay and contraindication criteria
- Height and weight

- Clinical laboratory investigations including resistance testing
- Study serology
- Assess menarchal and sexually active status

Post menarchal and sexually active girls and women and/or at the discretion of the site investigator ONLY:

- Pelvic examination, including
  - external genital examination (including genital warts assessment)
  - inspection of vagina and cervix
  - additional assessments as per local practice and/or clinically indicated (e.g. sexually transmitted infection screening samples, bacterial vaginosis, clinical Pap smear, etc.)
- Liquid based study cervical cytology and HPV DNA sample
- Vaginal microbiota swabs

MONTH 96/VISIT 13 (96 months from first vaccine):

- Assess ongoing consent/assent
- Health history update, Pap testing/treatment outside study, and any new/resolved health issues
- Update concomitant medication and exposure usage
- Review study blood work/genital sampling delay and contraindication criteria
- Height and weight
- Clinical laboratory investigations including resistance testing
- Study serology
- Assess menarchal and sexually active status

Post menarchal and sexually active girls and women and/or at the discretion of the site investigator ONLY:

- Pelvic examination, including
  - external genital examination (including genital warts assessment)
  - inspection of vagina and cervix
  - additional assessments as per local practice and/or clinically indicated (e.g. sexually transmitted infection screening samples, bacterial vaginosis, clinical Pap smear, etc.)
- Liquid based study cervical cytology and HPV DNA sample
- Vaginal microbiota swabs

5. Study Procedures in Detail

7.5.1 Informed Consent/Assent

Please refer to section 6.3 for detailed information on Informed Consent/Assent.

7.5.2 Medical History

General Medical Systems History

A review of health history including current medications will be conducted.

7.5.3 Height and Weight

Height and weight will be documented.

7.5.4 Clinical Laboratory Investigations

Clinical blood samples will be collected, handled, processed, and analyzed at site local laboratories as per local practices. At each visit in which clinical laboratory investigations are to be performed, results for the following laboratory tests will be collected as part of the study: Hgb, WBC, platelets, ALT, AST, ALP, bilirubin, BUN, serum creatinine, CD4, CB8 and HIV RNA quantitative PCR (viral load). Results of any Hepatitis B or C and resistance testing, including clade, will be updated whenever additional resistance testing is performed.

Clinical labs for study purposes may be collected within 3 months (+ or – 12 weeks) of the study visit date.

7.5.5 Study Blood Work

Ensure all blood tubes and cryovials are clearly labeled with the 5 digit PID#, collection date, and visit month. Study blood work must be collected within 24 hours of the study cervical cytology/HPV DNA sample.

7.5.6 Pelvic Examinations

Pelvic examinations will include:

- Inspection of external genital exam including a standardized assessment of genital warts using a tool provided by the Study Coordinating Centre,
- Speculum exam including clinical Pap smear (if clinically indicated),
- Clinical standard Pap tests will be collected, handled, and processed as per local practices. The clinical standard Pap test sample is to be collected PRIOR to study cervical cytology collection,
- If clinical Pap is not done at study visit, obtain the result of most recent clinical Pap performed,
- If clinically indicated, samples are to be collected for sexually transmitted infection screening samples, bacterial vaginosis, etc.
- Vaginal microbiota swabs

7.5.7 Study Genital Samples

Ensure samples are clearly labeled with the 5 digit PID#, collection date, and visit month. Cervical cytology/HPV DNA samples must be collected within 24 hours of the study blood work sample.

7.5.7.1 Cervical Cytology and HPV DNA

Cervical cells will be obtained using a broom shaped endocervical cytobrush. Insert brush into the endocervical canal until the smaller bristles meet the ectocervix. Press gently and rotate clockwise 5 times

to ensure adequate cell sampling. The brush is then immediately rinsed in the PreservCyt solution by pushing the brush into the bottom of the vial 10 times to force the bristles apart. To ensure all cells are released, swirl the brush vigorously. Securely seal the vial and discard the collection device.

Samples may be kept at ambient to 4 degrees Celsius at the local sites until recalled monthly by the Study Coordinating Centre. The samples will then be shipped to the Coordinating Laboratory for Cytology/HPV DNA (BCCDC) where the samples will be processed and forwarded to the appropriate lab for cervical cytology (Cervical Cancer Screening Laboratory of BC) and for HPV DNA testing (Coutlée lab).

HPV DNA types and cervical cytology results may not be available to sites until the end of the study. Clinical Paps are being done as per standard of care and follow up to abnormal cytology will be at the discretion of the Site Investigator in cases where the liquid based cytology samples are available.

7.5.7.2 **Vaginal Samples for Microbiota Analyses**

Vaginal samples should be collected during the speculum exam. Samples will be obtained using a Dacron swab. Remove study swab from kit and unscrew cap from red top tube. Insert swab into the vaginal canal and gently brush the swab against the surface of the vaginal wall. Rotate the swab clockwise approximately 3 times during collection to ensure adequate cell sampling. Remove swab and place into empty tube. Break off the swab shaft at the score even with the top of the tube. Align the shaft with the hole inside the tube cap and push down tightly over the shaft, sealing the tube. Screw red cap on. Repeat process with second swab. Refrigerate at 4°C and transfer to -20°C as soon as possible. Complete the sample collection log to indicate date and time swabs are transferred to refrigerator and/or freezer.

7.5.7.3 **Concomitant Medications and Exposures**

Concomitant medications for the purpose of this study are to include: antiretroviral therapy, other vaccines,; exposures will include: use of drugs of addiction, tobacco, and alcohol.

At each visit, subjects will be asked about their medication usage/exposures. Information collected as part of the original CTN 236 will be reviewed with the subject and any changes to medications will be documented. Information to be collected is to include: name of 'medication' (generic name or trade name for combination medications), medical indication, and total daily dose, route, start and

stop dates. PRN medications may be listed as PRN for total daily dose.

If a subject is on and off a treatment throughout the study, please ensure that the start and stop date indicate the time the subject was on and off treatment. Additionally if the dosages change a stop date should be entered for the previous dosage and a new entry made for the start of the new dosage.

For exposure to drugs of addiction, tobacco, and alcohol, use the most basic description, for example, THC, heroin, cocaine etc., tobacco, ETOH. For route, indicate whether smoked, snorted, injected, oral etc. For frequency, have subject estimate usage per day, week, or month.

7.5.7.4 Lower Urinary Tract Symptoms Survey

The objective of this survey is to further investigate the prevalence and impact of lower urinary tract symptoms in cohort participants above the age of 18. This survey is a one-time cross sectional measure comprised of 14 questions, to be administered at one study visit per participant; questions may be answered at any of the 6 possible study visits and will take 10 – 15 minutes to complete.

The survey is comprised of 14 questions and includes two validated instruments.[85] The Urogenital Distress Inventory 6 (UDI-6) asks questions about certain bladder symptoms and how much participants are bothered by them. The Urinary Incontinence Questionnaire 7 (UIQ-7), ask questions about how much these symptoms affect participants' activities of daily living. This study questionnaire concludes with one supplementary question regarding prevalence of urinary tract infections in the past year. Inclusion of this survey will provide a greater understanding of gynaecological health in this cohort and speak to any relation between HPV infection and urogenital distress.

8.0 MANAGEMENT OF CERVICAL CYTOLOGIC ABNORMALITIES

All participants (except those pre-menarchal and not yet sexually active) will undergo clinical standardized Pap smear testing as per local site standards. Cytologic abnormalities will be managed according to local algorithms. Management of cervical cytologic abnormalities will be decided by the Site Investigator and may be based on the clinical standardized Pap smear or study cervical cytologies when available. It is suggested to manage abnormal results based on the higher grade of dysplasia.

Participants who have high grade or persistent low grade changes will be referred for follow-up evaluation by colposcopists involved with local HIV treatment centres. After obtaining consent to release medical records, all colposcopic, colpophotographic and histopathologic (biopsy) results will be added to the study record; slides from biopsies will be requested by the BC Cancer Agency for central confirmation of histopathologic diagnoses. Invasive cancers diagnosed during the study will be carefully documented and recorded once local, appropriate clinical management is provided. As in previous multicentre studies of high risk HIV positive women, we acknowledge variation in local colposcopic and treatment algorithms and anticipate imperfect compliance with referral for colposcopy; similarly, cervical disease endpoints (squamous intraepithelial neoplasia or dysplasia) in this study are cytologically and/or histologically defined as appropriate. Histopathology will be reported as cervical intraepithelial neoplasia grades, 1, 2 or 3 (CIN 1, 2 or 3), or carcinoma in situ.

9.0 SERIOUS ADVERSE EVENTS

The investigator is responsible for documentation and reporting of any Serious Adverse Event that results in death.

1. Reporting/ Time Period

All SAEs resulting in death occurring during the study must be recorded and reported to the Study Coordinating Centre whether or not they are considered vaccine-related. Sites should continue to use the SAE reporting forms utilized in CTN236.

SAE reports will be faxed to Dr. Deborah Money at the **Study Coordinating Centre within 24 hours of discovery or notification to the study centre of an SAE resulting in death and within 48 hours of discovery to the Study Data Management Centre (CTN).** The Study Coordinating Centre will promptly notify Health Canada as appropriate. Site investigators are obligated to notify their REB as per local reporting requirements.

SAE Notification:
Dr. Deborah Money (fax#) 604-875-3895 (24 hours)
AND CTN (fax#) 604-806-8005 (48 hours)

The Study Coordinating Centre will acknowledge receipt of the faxed SAE form.

Site Investigators should also report SAEs to their provincial adverse event reporting centre as would occur when administering any routine licensed vaccine.

10.0 PREGNANCY

All pelvic examinations, including Pap testing and study sampling will be continue during pregnancy; however, an Ayre spatula will be used to collect cervical samples in place of the cytobroom until 3 months post resolution of the pregnancy.

11.0 SUBJECT COMPLETION AND WITHDRAWAL

Investigators or their delegates will attempt to contact those study subjects who do not return for study visits. Study personnel will make at least three attempts to contact the subject using various communication means. Each attempt should be documented in the subject source documents.

A subject completing three annual visits up to and including Month 96/Visit 13 will be considered to have completed the study.

Withdrawals will not be replaced. All withdrawals will be documented including the date of the withdrawal and the reason.

12.0 LABORATORY METHODS AND ANALYSES

Please consult the Research Laboratory Manual for detailed information on the requirements for supplies, collection, and processing, and shipping of laboratory specimens.

Blood Testing

Whole blood by venous route is to be collected observing aseptic conditions into one 10 mL red top Serum Vacutainer (non-heparinized, non-EDTA, non-serum separator) tube. Specimens should be placed upright and allowed to clot for 30 to 60 minutes. After the sample has clotted is should then be refrigerated at 2-8 degrees Celsius until centrifuged. Samples are to be centrifuged within 24 hours of collection and according to manufactures specification to allow for complete separation of serum. Serum is to be aliquoted using a plastic pipette into 3 cryovials, each containing a minimum of 1.0 mL and then frozen between -60 and -80 degrees Celsius at the investigative site until recalled by the Study Coordinating Centre. Samples will then be shipped frozen to the Coordinating Laboratory for serum samples.

The Coordinating Laboratory will arrange for transportation of the samples to the individual laboratories performing serotesting, specifically , 1) BC Centre for Disease Control, Virology research laboratory, Vancouver, BC, and 2) Merck & Co., Wayne, Pennsylvania and 3) to remain at the BC Women's Research Laboratory at BC Women's Hospital & Health Centre.

12.1.1 HPV Serotesting

Each serum sample will be divided into 3 aliquots with one going to the BCCDC Virology research laboratory, Vancouver, BC, the second being sent to the Merck laboratory for in house assay assessments, and the third being sent to the BC Women's Research Laboratory for storage. The Merck laboratory will be conducting the immune assays on the same platform as those performed for the pre-licensure published antibody.

12.1.2 Merck laboratory Serotesting Methods

Serum anti-HPV 6, 11, 16, and 18 immunoglobulin levels will be measured using a competitive Luminex immunoassay (cLIA), and reported in arbitrary units (milli-Merck Units per millilitre or mMU/ml) relative to the standard curves generated for each individual HPV type.[86] The HPV cLIA uses yeast-derived HPV L1 6, 11, 16 and 18 VLPs that have been covalently conjugated to Luminex microspheres. The type specific HPV-VLP antibody responses are associated with specific Luminex microspheres that are identified by their distinct fluorescent dye spectral properties. Antibody titers are determined in a competitive format, where known, with type-specific labeled neutralizing antibodies for each type competing with patient serum antibodies for binding to conformation-sensitive neutralizing epitopes on the VLPs. For each HPV type, relative inhibition is compared to a pooled standard reference serum. Seropositivity will be defined in this assay as > 20 mMU/ml for HPV 6, 16 mMU/ml for type 11, 20 mMU/ml for 16 and 24 mMU/ml for 18. Of note, lower limits of detection for this assay are 8.0, 8.0, 12.0, and 8.0 mMU/ml for 6, 11, 16 and 18 respectively.[67]

12.1.3 HSV2 Serology Testing

Samples will be sent to the BCCDC Virology research laboratory, Vancouver, BC for analysis. The HerpeSelect ELISA (Focus Technologies) will be used but will employ a high cutoff index value of 3.5, which has been shown to offer improved specificity in the setting of HIV co-infection.[87,88,89] Previously stored blood will be pulled as per protocol for baseline testing and at each study visit, participants will have serum stored for batched testing of HSV type-specific antibody. After baseline determination of serostatus for all participants, follow-up testing will be performed only on previously seronegative participants to assess for interval seroconversion.

13.0 Genital sampling

All PreservCyt samples will be sent monthly by the local sites to the Coordinating Laboratory for Cytology/HPV DNA. Samples will be re-suspended in the Preservcyt and a 4 ml aliquot will be taken and sent to the Coutlée Laboratory for analysis of HPV DNA. The remainder of the sample will be sent to the Cervical Cancer Screening Laboratory of BC for cervical cytology.

1. HPV DNA testing – Coutlée Laboratory

The 4 ml endocervical samples in PreservCyt will be centrifugation at 13000xg for 15 min at 22°C, the supernatant will be discarded, the cell pellet will be left to dry and re-suspended in 300 µl of 20 mM Tris buffer, pH 8.3.[63,64] DNA will be purified with Master pure.[65] ß-globin and HPV DNA amplification and typing. Extracted DNA will be tested in batches for ß-globin with PC04 and GH20 to screen for inhibitors or inadequate quantities of cellular DNA and for HPV DNA with previously described PGMY primers.[66,67] Samples negative for ß-globin are considered

inadequate. PCR L1 consensus tests are well standardized and have been validated on large numbers of clinical samples, as reviewed by members of this team.[68,69] HPV DNA will be first amplified with PGMY09-PGMY11 and typed with the quality-controlled Linear array assay commercialized by Roche Molecular Systems.[66,67,70-73] This specific PCR assay reliably detects 10 HPV DNA copies.[66,71] This test permits testing and typing for 37 genital types of HPV (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51-59, 61, 62, 64, 66-73, 81, 82, 83, 84, 89 and IS39 (a subtype of HPV 82)). The genotypes relevant for this proposal including 6, 11, 16 and 18 are all detected by these specific PCR protocols. The Coutlée laboratory has extensive experience with PCR for HPV detection. [66-68,70-74,74-76, 78-80 ,82,83] and has participated in the evaluation and validation of the Linear array and PGMY09-PGMY11 primers. [66,67,70,71 ,83] Weak (10 HPV18 DNA copies), and strong positive HPV controls, will be included in each amplification run as internal control and precautions to avoid contamination will be in place at all times.[68,74,76,78,80,82]

2. **Cervical Cytology – Cervical Cancer Screening Laboratory of BC**
The PreservCyt vial will be processed for cytologic evaluation following the ThinPrep protocol (Cytyc Incorporated). Slides will be read by cytotechnologists at the Cervical Cancer Screening Laboratory (CCSL) of BC. Reporting rules for normal and abnormal results will be the same as for routine specimens processed at the CCSL, a facility that processes all cervical smears performed in British Columbia (up to 600,000 per year). Standard reporting of Pap smears will be according to the Bethesda criteria e.g. low grade and high grade squamous intraepithelial neoplasia (SILs)

3. **Vaginal Microbiota Analyses – Hill Lab, University of Saskatchewan**
Vaginal samples will be collected for microbiome analysis as per the VOGUE Emerging Team Grant protocol (CIHR FRN 108030; PI D Money). Samples will be transported to the Hill laboratory at the University of Saskatchewan, where total DNA will be extracted from vaginal swabs using the MagMAX magnetic DNA binding platform (Applied Biosystems). Chaperonin 60 (cpn60) amplicons130 will be then prepared as previously described utilizing multiplexed ID sequences (MID) to pool samples during sequencing.[90] Sequencing will be performed on the Roche GS Junior pyrosequencer in the Hill lab. Raw sequence data will be processed initially using the standard on-rig software tools for quality control and filtering. Quality filter-passed read data will then be analyzed using the mPUMA bioinformatics pipeline developed by the Hill lab.[91]

14.0 TRANSPORTATION OF LABORATORY SAMPLES

All shipments of <u>serum samples</u> and <u>vaginal microbiota swabs</u> are to be shipped frozen and prepared by qualified individuals ensuring that all shipments are packed and shipped according to IATA regulations and are to be packed using dry ice.

All shipments of liquid based cytology are to be shipped ambient and prepared by qualified individuals ensuring that all shipments are packed and shipped according to IATA regulations.

All samples are to be clearly labeled with subjects' 5 digit PID#, date of collection, and visit month.

All shipments must have an accompanying biological shipment listing, with a copy being retained at the site. A biological shipment listing template will be provided to the sites by the Study Coordinating Centre.

Prior to the shipment of any samples, the sites must inform the Study Coordinating Centre regarding the date of shipment to ensure that there is someone at the Coordinating Laboratory to receive the shipment. Confirmation must be received from the Study Coordinating Centre prior to the shipment of any samples. Sites should plan to ship Monday through Wednesday to ensure that samples arrive within 72 hours of packaging to ensure that thawing does not occur before processing.

The Coordinating Laboratories will arrange for shipment of all samples (serum, PreservCyt, microbiota swabs) to the individual laboratories performing the analysis/testing.

15.0 DATA SAFETY MONITORING BOARD/COMMITTEE

The established CTN data safety monitoring board (DSMB) will be used for this study with additional representation by pediatrics and gynecology-oncology. The DSMB will provide oversight and monitoring of the conduct of the study to ensure the safety of the subjects. Additionally, the DSMB will ensure the validity and integrity of the study data. The DSMB meets twice a year.

16.0 STATISTICAL ANALYSIS

1. Statistical Analysis Plan

Demographics: Characteristics of the study population will be described with frequencies and proportions for categorical variables, means and standard deviations for normally distributed continuous variables and medians and interquartile ranges for skewed continuous variables.

2. Primary Analysis

For each of the four vaccine containing HPV types, antibody response will be summarized using GMTs calculated for age specific groups within the HIV-positive cohort. Specific age groupings have been chosen to permit age matched HIV comparisons.

a) Antibody response for subjects aged 9-13 and those aged 16-26 will be compared to the individual patient level data from an HIV-negative cohort 86 using Wilcoxon rank-sum tests. The age adjusted fold-difference in GMT

between HIV-positive and HIV-negative populations will be calculated using generalized linear models. For women over age 26, the type specific HPV GMT will be compared to GMTs in the published literature using a sign test.

b) Within the HIV-positive cohort, predictors of decline from peak antibody response among HIV-positive girls and women will be determined using generalized linear models. Potential covariates to be considered in these models are age, HSV-2 status, substance use, CD4 count, HIV viral load and antiretroviral status at the time of first vaccination, and CD4 nadir.

c) Longitudinal analyses will be conducted to describe the effect of HIV on antibody response at the visits at 36, 48, 60, 72, 84, and 96 months post first vaccine dose. Generalized estimating equation (GEE) models with a unity link will be used to adjust for correlation among repeat observations within study participants. Individual level data on HIV-negative girls and young women will be available at 36, 48, 60, 72, 84 and 96 months post first vaccine dose. After adjusting for age, an interaction between HIV and time since first dose will be included in the model to test for a difference in the degree of waning of response in addition to the strength of the initial response. GEE models will also be fit for the HIV-positive girls and women alone to examine the associations.

d) Predictors of HPV antibody seroresponse in our study population will be identified using logistic regression. This analysis is considered to be of vital importance as other vaccines have been shown to not be effective in subgroups of HIV positive individuals and could permit targeting of the proper population allowing for cost-effective use of this vaccine. The primary predictor of interest is CD4 count and justification for this is based on hepatitis B vaccination data described above. The secondary predictors of interest will include the variables: age, presence and number of strains of HPV, HIV viral load, use of HAART, use of injection drugs, and ethnicity. These predictors were chosen as ones that make the most clinical sense for potent impact on vaccine antibody response.

3. Exploratory Analyses

HSV analyses will employ linear or logistic regression models as appropriate, with HSV-2 serostatus as a time- updated primary predictor variable. To address these issues, we will quantify the impact of both incident (acquired within the past year) and prevalent (present at the time of diagnosis) HSV-2 co-infection on each of the primary HPV-related outcomes as described in preceding section (GMT, breakthrough HPV infections, genital tract dysplasia, and external genital warts).

Vaginal Microbiota Analysis. The output of the bioinformatics pipeline, including inventory of unique operational taxonomic unit (OTU) sequences, their taxonomic lineage and abundance, will be used to visualize and characterize the vaginal microbiota. Using various community-level analyses available as packages in R: 133

notably the BiodiversityR, 134 vegan, 135 and ALDeX2136 packages, we will apply statistical and graphical techniques to identify patterns of bacterial diversity that are associated with HPV infection and disease. Specifically, we will use compositional analysis as implemented in ALDeX2 to test for relationships between specific bacterial taxa and HPV infection and cervical dysplasia in addition to other clinical variables at each time point. Taxa thus identified will be examined for changes in relative abundance across time using mixed-effects models to control for multiple measures on each subject, and comparing to HPV infection status, HPV type and other clinical and HIV correlates. We will also use hierarchical clustering methods to determine if the bacterial communities form identifiable and robust clusters, with particular species occurring more often together. We can then test if these clusters are associated with HPV infection, burden of infection (single vs. multiple types) and evidence of cervical disease using standard statistical tests such as logistic regression and ANOVA. Finally, we will use mixed-effects models to test for changes in bacterial diversity and species richness that are associated with HPV infection, burden of infection (i.e. infection with multiple HPV types) and evidence of cervical disease while controlling for repeated measures on each subject.

HIV and Lower Urinary Tract Symptoms Analysis. Descriptive statistics will be used for baseline patient characteristics. Logistic regression analysis will be performed to determine the effect of: severity of HIV (CD4 count, viral load, AIDS criteria met), current HIV medications, time since HIV diagnosis, and vaginal microbiome on lower urinary tract symptoms, while also considering the effects of age, parity, ethnicity, sexual practices (from various questions in the HPV in HIV questionnaire), history of STI, history of UTI, smoking status, alcohol use, drug use.

17.0 DATA MANAGEMENT

Data management will be performed by the CIHR Canadian HIV Trials Network (CTN).

Source document data will be transferred to a case report form by study personnel at the investigative sites. The participant identification number (PID) and a unique 3 letter code that was assigned in CTN 236 will be used to label the case report form and all study samples.

Data will be entered into a relational database with pre-programmed quality assurance checks. Queries will be resolved with the site study coordinator, with appropriate documentation. Data edits will checked by the CTN data manager before being added to the master database. Backup copies of the database will be made daily and stored securely at a second location. No personal identifiers will be included in the database. Analysis will be conducted using statistical software (e.g. SAS or SPSS).

All personal information pertaining to participants will be securely stored at sites in a locked room, under the supervision of the site study coordinator(s) and accessible to study staff only. Where electronic databases are used as mechanisms for subject tracking etc. these databases will be password protected and available only to specified study staff and site investigators.

18.0 ADMINISTRATIVE MATTERS

1. Protocol amendment procedure

No modification of this protocol will be allowed unless discussed and approved with the Study Coordinating Centre and a filed and approved change to the protocol is made with local REBs.

Any administrative changes or amendments to this protocol will be adhered to by all participating centres and will apply to all subjects. Documentation of the amendments and REB certification will be maintained at each investigative site and are required prior to implementation.

2. Records Retention

Data and study documents (all sites) will be stored securely for 25 years, after which they will be destroyed in keeping with privacy and confidentiality regulations and guidelines.

All personal health information pertaining to subjects will be securely stored in a locked area, under the supervision of the site study coordinator(s) and accessible to study staff only. The location of storage of the study files must be documented and revised if the location changes. The Study Coordinating Centre must be aware of the storage location at each site and changes to the location must be provided in writing.

3. Clinical Study Report

Interim study progress reports, as well as the final report, will be submitted to the Canadian Institutes of Health Research.

4. Publication Plan

Manuscripts of the study findings will be prepared for submission to peer reviewed publications. Study findings will also be presented at appropriate scientific meetings.

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