Protocol: Long-term Human Papillomavirus Effectiveness and Immunity in Rwandan Women Living With and Without Human Immunodeficiency Virus

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Long-Term Human Papillomavirus Vaccination Effectiveness and Immunity in Rwandan Women Living with and Without Human Immunodeficiency Virus

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ACRONYMS

Adenocarcinoma *in situ* (AIS)
Age Standardized Rates (ASR)
Albert Einstein College of Medicine (Einstein)
American Society for Colposcopy and Cervical Pathology (ASCCP)
Anal Intraepithelial Neoplasia (AIN)
AIN Grade 2 (AIN2)
AIN Grade 3 (AIN3)
AIN2 or More Severe Diagnoses (AIN2+)
AIN3 or More Severe Diagnoses (AIN3+)
Antiretroviral Therapy (ART)
Audio Computer-Assisted Self-Interview (ACASI)
Central Africa International Epidemiology Databases to Evaluate AIDS (IeDEA)
Cervical Intraepithelial Neoplasia Grade 3 (CIN3)
Cervicovaginal microbiome (CVM)
CIN Grade 2 (CIN2)
CIN Grade 3 (CIN3)
Confidence interval (CI)
Formalin-fixed paraffin-embedded (FFPE)
Frederick National Laboratory of Cancer Research (FNLCR)
Generalized estimating equations (GEE)
Geometric mean titers (GMT)
High-resolution anoscopy (HRA)
High-risk HPV (hrHPV)
HIV-negative (HIV[-])
Human Immunodeficiency Virus (HIV)
Human Papillomavirus (HPV)
Infrared coagulation (IRC)
Invasive Cervical Cancer (ICC)
Loss to follow-up (LTFU)
Ministry of Health (MoH)
Next-generation Sequencing (NGS)
U.S. National Cancer Institute (NCI)
Participant Identification Number (PID)
People Living with HIV (PLWH)
REDCap (Research Electronic Data Capture)
Research for Development (RD Rwanda)
Rwanda Military Hospital (RMH)
Sub-Saharan Africa (SSA)
University of Rwanda (UR)

U.S. Centers for Disease Control and Prevention (CDC)
Virus-like particles (VLPs)
WLWH (Women Living with HIV)
World Health Organization (WHO)

Table of Contents

EXECUTIVE SUMMARY

Cervical cancer is the 4th most common cancer and cause of cancer-related death in women globally; in many lower-resource settings, especially sub-Saharan Africa (SSA), it is the most common. Virtually all cervical cancer and precancer are caused by 12-15 high-risk human papillomavirus (HPV) types. HPV16 causes approximately 55-60% and HPV18 causes approximately 10-15% of cervical cancer while the remaining 12 HPV types cause the remaining 25-30% of cervical cancer. High-risk HPV, predominately HPV16, also causes most anal, vulvar, vaginal, and penile cancers and a significant proportion of oropharyngeal cancers. Prophylactic HPV vaccines have been developed and have been shown to be nearly 100% protective against incident HPV infection and related abnormalities in the general population. However, the evidence for the effectiveness of prophylactic HPV vaccines in women living with human immunodeficiency virus (HIV) (WLWH) is less clear. HIV infection increases the risk of cervical cancer due to an impaired immune response to HPV.

Rwanda is a high-burden cervical cancer country where the prevalence of HIV is 3.7% among adult women, with higher HIV prevalence among young women (1). In 2011, Rwanda implemented a national HPV vaccination program with Gardasil®, which protects against HPV16 and HPV18, the two HPV types that cause ~70% of cervical cancer, and HPV6 and HPV11, the two types that cause ~90% of anogenital warts (HPV6/11/16/18). Their program has achieved >90% coverage of the target population, primarily girls aged 12 years, annually. The implementation of a highly successful HPV vaccination program and the high prevalence of HIV, in addition to the research and medical capacity that Albert Einstein College of Medicine (Einstein) has helped to build at the Rwanda Military Hospital (RMH), Research for Development (RD Rwanda) and University of Rwanda (UR), makes Rwanda the ideal locale to study the long-term effects of HPV vaccination in WLWH.

To answer questions about HPV vaccine effectiveness and immunity in Rwandan WLWH, collaborators at Einstein, RD Rwanda, RMH, and UR will conduct an observational study of WLWH and HIV-negative (HIV[-]) women who did (birth cohorts 1996 and later) and WLWH who did not (birth cohorts before 1996) receive HPV vaccination through the national vaccination program. We will compare cervicovaginal, anal, and oral prevalent and 6-12 month persistent HPV6/11/16/18 infections in HPV-vaccinated and -unvaccinated WLWH and HPV-vaccinated and -unvaccinated HIV[-] women (n=757 per group; 4 groups; 3,028 women total). We will also compare the HPV immune response in at least 548 HPV-vaccinated WLWH to at least 548 HPV-vaccinated HIV[-] women and the impact of switching from 3 doses to 2 doses of Gardasil® in 2015. Finally, we will investigate the risk factors, including the cervicovaginal microbiome, for prevalent and 6-12 month persistent HPV infection in young WLWH and HIV[-] women. Our long-term goal is to establish a cohort of WLWH in whom we can examine the long-term effectiveness of HPV vaccination in WLWH now and in the future. This contribution is significant as it will establish the population effectiveness of HPV vaccination in WLWH living in SSA, the

group of women at the highest risk of cervical cancer, for which there is a dearth of evidence. The proposed research is innovative as it leverages and expands the local research and medical capacity in Rwanda to examine one of the critically unanswered questions about HPV vaccine effectiveness in the context of the World Health Organization's (WHO) call for the elimination of cervical cancer.

I.INTRODUCTION

I.A. Epidemiology and Natural History of Human Papillomavirus (HPV) and Cervical Cancer

Cancer of the uterine cervix, invasive cervical cancer (ICC), with an estimated annual incidence of 570,000 cases and 311,000 related deaths, is the 4th most common female cancer and cause of cancer-related mortality in females globally (2). The introduction of effective Papanicolaou (Pap)-based (cytology) screening in some, mostly high-income, countries in the mid- to late-20th century has led to a steady decrease in cervical cancer incidence and mortality in those countries (3, 4). More than 80% of all cervical cancer occurs in low- and middle-income countries (2), where high-coverage, effective cytology-based screening has never been successfully implemented (3, 4).

Africa, specifically SSA, suffers the greatest burden of ICC. Approximately one-fourth of all ICC and ICC-related deaths globally occur in Africa. More than 90% of ICC in Africa occurs in SSA, which has an ICC incidence age-standardized rate (ASR) of 34.8 per 100,000 and mortality ASR of 24.1 per 100,000 (2). Rwanda has ICC burden similar to SSA with incidence ASR of 31.9 per 100,000 and mortality ASR of 24.1 per 100,000 (4). In addition to a lack of capacity to implement Pap-based screening there, women living in SSA suffer from many other infectious disease epidemics, including human immunodeficiency virus (HIV). Cervical cancer was included as an acquired immunodeficiency syndrome (AIDS)-defining disease in adolescents and adults in 1993 (5-7).

WLWH have a significantly elevated risk of cervical cancer (8, 9), due to an impaired immune response to HPV, compared to HIV-uninfected (HIV[-]) women. People living with HIV (PLWH) are also at an increased risk of other HPV-related malignancies, notably anal and oropharyngeal cancers (8, 10). HIV infection is an important contributor to the unequal burden of cervical cancer in SSA due to its high prevalence compared to the rest of the world (11-13). Many SSA countries have a HIV prevalence among those aged 15-49 years of >5% and even >20% (14). In Rwanda, it is estimated that 3.0% (1) of the adult population are living with HIV with a prevalence of 1.8% among women aged 20-24 and 3.8% among women aged 25-29 years (15).

Persistent cervical infections by high-risk HPV types cause virtually all ICC and its immediate precursor lesions, e.g., cervical intraepithelial neoplasia grade 3 (CIN3) and adenocarcinoma *in situ* (AIS), everywhere in the world (16, 17). HPV causes most anal and vaginal cancer and a significant proportion of vulvar, penile, and oropharyngeal cancers (18). HPV16 is the most important, responsible for approximately 55-60% of ICC (19). HPV18 is the next most important, responsible for approximately 10-15% of ICC, including 30% of adenocarcinoma of the cervix (19), which is on the rise in Western Countries (20, 21). Together, HPV16 and HPV18

account for approximately 70% of ICC and the same ~15 high-risk HPV types account for ~99% of ICC everywhere in the world (19).

The natural history of HPV and cervical carcinogenesis can be represented by a simple, causal schema of four, reliably measured stages (22): 1) HPV acquisition, 2) HPV persistence, 3) progression to precancer (CIN3/AIS), and 4) ICC. The key step in cervical carcinogenesis is overt, measurable HPV persistence, which even after a year or two strongly predicts the development of cervical precancer and cancer (i.e., CIN3 or more severe diagnoses) (CIN3+) (23). HIV co-infection has a profound impact on the natural history of HPV, thereby increasing the risk of ICC. HIV co-infection increases the 1) likelihood of cervical and anal HPV persistence and 2) likelihood of cervical and anal HPV infections progressing to precancer (24, 25). Incident HIV infection also significantly increases the risk of newly detected cervical and anal HPV infection, presumably due in part to immune dysregulation resulting in increased persistence of even transient infections (prevalence = incidence * duration) and/or and re-activation of a latent/well-controlled HPV infection (25).

Meta-analyses of HIV/AIDS cohorts reported a 6-fold increased incidence of cervical cancer compared to the general female population/HIV[-] women (26). Unexpectedly, despite the introduction of combined anti-retroviral therapy (ART) and concomitant immune reconstitution, the incidence of the HPV-related malignancies, notably cervix and anal carcinomas, has not decreased as they have for other malignancies (e.g., Kaposi sarcoma and specific types non-Hodgkin lymphoma i.e., Burkitt Lymphoma, Immunoblastic Lymphoma, and primary lymphoma of the brain) and may have increased. This increase is perhaps the result of HIV-infected patients living longer (27, 28), long enough to develop HPV-related cancers (29, 30).

I.B. Prophylactic HPV Vaccination

The discovery of high-risk HPV (hrHPV) infections as the necessary cause of cervical cancer has led to revolutionary advances in cervical cancer prevention, including the development of prophylactic HPV vaccines for primary prevention. Current prophylactic HPV vaccines are based on the self-assembly of recombinantly expressed L1 protein in cell lines into virus-like particles (VLPs) that resemble native viral capsids but without the viral genome necessary for viral replication.

The first generation of HPV vaccines, Gardasil® (Merck & Co, Kenilworth, NJ, USA) (31) and Cervarix™ (GlaxoSmithKline, Wavre, Belgium) (31), targeted HPV16 and HPV18 (HPV16/18). Gardasil® also targets HPV6 and HPV11 (HPV6/11), non-high-risk HPV types responsible for 90% of anogenital warts (*Condyloma acuminata*). The next generation HPV vaccine, Gardasil® 9 (Merck & Co) (32), targets HPV31, 33, 45, 52, and 58 in addition to HPV6, 11, 16 and HPV18 and is predicted to prevent ~90% of cervical cancers globally.

Given that HPV vaccination is only prophylactic and not therapeutic, the ideal timing of HPV vaccination is prior to sexual initiation and exposure to HPV i.e., HPV-naïve women. On a population level, this can be achieved by vaccinating a few years before the population median age of sexual initiation. As the median age of sexual initiation is typically 15-17 years in many populations, the World Health Organization (WHO) recommends vaccination programs to target 9-13-year-old girls (33). With older cohorts of women, prophylactic vaccines are equally efficacious but less effective i.e., fewer women benefit from HPV vaccination, resulting in lower effectiveness and cost-effectiveness, because some women have already been infected by HPV (34-36). Based on immunogenicity data, WHO (37) and the U.S. Centers for Disease Control and Prevention (CDC) (38, 39) now recommend two-dose schedules for those under the age of 15 years and three doses for those 15 years and older for all HPV vaccines.

There are ample data demonstrating efficacy and population effectiveness of Gardasil® against HPV6, 11, 16, and 18 (HPV6/11/16/18) infections, anogenital warts (40), related high-grade cervical abnormalities (41), and now even cancer in immune-competent populations (41). Several countries, e.g. Australia (42-48), Scotland (49), Denmark (50), and the U.S. (51-53), were early adopters of HPV vaccination and have documented reductions in HPV infections and -related diseases and abnormalities. A meta-analysis on the impact of HPV vaccination found reductions in anogenital warts, HPV infections, CIN grade 2 (CIN2) or more severe diagnoses (CIN2+) among girls and women, and on anogenital warts diagnoses among girls, women, boys, and men (40). A report from Finland provided the first evidence that HPV vaccination prevents cervical cancer (41). Recent reports from Finland, Sweden, and Denmark now provide real-world evidence that HPV vaccination prevents cervical cancer (41, 54, 55).

I.C. Prophylactic HPV Vaccination in People Living with HIV (PLWH)

Data on HPV vaccine efficacy and effectiveness data are lacking for WLWH (8, 9). To date, most studies of HPV vaccine in WLWH focused on immunogenicity and safety. Generally, HPV vaccination in PLWH has been well tolerated, safe, and resulted in good immune responses. A study of Gardasil® of children living with HIV and another study of WLWH reported high seroconversion to Gardasil (56, 57). The same study in children living with HIV showed high 4-year persistent HPV type-specific immunity (58) and immune memory cells as well as a significant increase in, and persistence of, antibody titers following a 4th dose (59).

Studies have noted an impact of HIV disease status (CD4 counts and viral suppression) on the immune responses to HPV vaccination. Studies have reported lower seroconversion and antibody titers in WLWH not taking (vs. taking) antiretroviral therapy (ART) and with lower (vs. higher) CD4 counts (57, 59). Similarly, another study found peak antibody titers to be 2- to 3-fold higher in mid-adult WLWH with full HIV viral suppression compared to those not suppressed (60). Interestingly, higher anti-HPV18 titers but similar anti-HPV16 titers were reported in response to

HPV vaccination by Cervarix® compared to Gardasil® in PLWH but the differences in the former was primarily due to differences in the immune responses in the WLWH (61).

The few studies of HPV vaccine effectiveness in WLWH provided promising but inconclusive data on immunogenicity. However, these completed studies of HPV vaccination in PLWH have not documented any measurable efficacy or effectiveness, and notably long-term effectiveness. Immunogenicity studies in WLWH have been of insufficient sample size to address efficacy/effectiveness. Studies of HPV vaccination in select PLWH at high risk of anal cancer have been limited in sample size because of the high HPV anal exposure (and likely misclassification of exposure) to targeted HPV types prior to enrollment (62) so that few truly incident events could be observed. Of those studies that did have endpoints, one recent trial of Gardasil® in PLWH aged 27 and older concluded that "This double-blind, randomized trial did not find a benefit from HPV vaccination to prevent anal HPV infection or anal high-grade squamous intraepithelial lesions" and favorable but inconclusive benefit for protection against oral HPV (63). Another study reported 5-fold higher incidence of abnormal cervical cytology among perinatally HIV-infected vs. HIV-exposed, uninfected youths (64).

ClinicalTrials.gov (65) reports several ongoing studies (i.e., enrollment listed as open) of assessing HPV vaccination in PLWH but none address prophylactic efficacy or long-term effectiveness. Several new projects are being launched as part of the "Prevention of HPV-related Cancers in HIV-infected Individuals: United States-Latin American-Caribbean Clinical Trials Network: Partnership Centers" (66) have HPV vaccination studies but are limited in scope and will not provide long-term effectiveness for HPV vaccination on cervicovaginal or anal HPV infection in WLWH.

The paucity of HPV vaccine effectiveness data in WLWH is especially a concern for the prevention of cervical cancer in SSA where cervical cancer is the most common cause of cancer death in women. Almost one quarter of the global burden of cervical cancer occurs in SSA and an estimated 20% of cervical cancer in SSA is attributable to HIV coinfection (26). Evidence of the protective effects of HPV vaccination in SSA WLWH are needed, especially since most long-term public health planning to address cervical cancer in SSA depends on the unproven effectiveness of these vaccines.

I.D. HPV Vaccination Program in Rwanda

In 2011-13, Rwanda, through a donation from Merck, launched a national HPV vaccination program. In 2011, over 92,000 girls in primary school grade six (~12 years old) were vaccinated with three doses of Gardasil®. During 2012 and 2013, a catch-up vaccination program targeted girls in secondary school grade three (~15 years old) (67). In 2014, HPV vaccination was supported by GAVI (68) and reverted to vaccinating 12-year-old girls (69). In 2015, Rwanda switched from 3 doses to 2 doses, 6 months apart, for vaccinating 12-year-old girls. In all years, Rwanda achieved ≥90% annual coverage with the recommended number of HPV vaccine doses

in the target population (67, 68, 70, 71). Thus, Rwanda is one of the earliest and most successful adopters of HPV vaccination globally. A summary of the HPV-vaccinated population is shown in **Table 1**.

Table 1. HPV vaccination data for Rwanda

Year	N (HPV Vaccinated)	Approximate age at vaccination (years)	Birth year of vaccinated	Approximate age in 2021/2023 (years)	N HPV-Vaccinated WLWH (estimated)
2011*	88,927	11-13	1998-2000	21-23/23-25	1,600
2012*	134,745	11-16	1996-2001	20-25/22-27	2,430
2013*	134,775	11-16	1997-2002	19-24/21-26	2,430
2014*	142,192	12	2002	19/21	2,560
2015**	106,522	12	2003	18/20	1,920
2016**	120,953	12	2004	17/19	2,180
2017**	120,594	12	2005	16/18	2,170
Total	848,708				15,290

*three doses of Gardasil®; **two doses of Gardasil®

Data provided by the Rwanda Ministry of Health

II.RATIONALE

In 2018, WHO called for the elimination of cervical cancer as a public health problem (72). To accomplish this goal, WHO put forth an ambitious strategic plan (73) that includes 90% of girls fully vaccinated against HPV by 15 years of age. Yet, we do not know if or how well HPV vaccination works in PLWH. Recent national and international guidelines have highlighted the lack of direct evidence of HPV vaccination in PLWH. Studies have demonstrated that HPV vaccines are safe and immunogenic in various groups of PLWH. Some studies demonstrated antibody levels lower in individuals with HIV compared to those without HIV, but the clinical significance of this observation is unknown (60, 74, 75).

Given the high prevalence of HIV in SSA, it will be important to document both long-term effectiveness and immunity. Such data will inform strategies to optimize the impact of HPV vaccination in PLWH.

Rwanda is the ideal locale to answer questions about the long-term effectiveness and immunogenicity of Gardasil® in WLWH. The country has experienced nearly complete national coverage with Gardasil® since more than 10 years ago, starting in 2011, so predominately HPV-vaccinated and unvaccinated WLWH are easily recruited based on birth year. Rwanda has excellent national vaccination records so that retrospectively the small number of participants misclassified by HPV vaccination status based on birth year subsequently can be correctly categorized. The prevalence of HIV in Rwanda is 2.7% among 15-30 year olds (1). The excellent HIV care program allows for easy and efficient recruitment and following of WLWH in an observational study of HPV vaccination impact. Rwanda is part of the Central Africa International Epidemiology Databases to Evaluate AIDS (IeDEA) consortium whose infrastructure can be leveraged to recruit and retain WLWH women in the proposed observational study. The Rwanda / US collaborative research group has a well-established, long-term, and highly productive clinical and research presence in Rwanda, representing a unique opportunity to establish a prospective cohort study of the population effectiveness of HPV vaccination in WLWH (**Section V**).

III. SPECIFIC AIMS

We propose the following Specific Aims for our study of HPV vaccination in Rwandan WLWH:

1. To measure population effectiveness of prophylactic HPV vaccine in reducing cervicovaginal, anal, and/or oral prevalent and 6-12 month persistent infections by HPV6/11/16/18; and

2. To quantify, and examine the determinants of, long-term antibody (into young adulthood) responses to HPV vaccination

Exploratory Aim: Conduct a natural history study to investigate determinants, including cervicovaginal microbiome (CVM), of short-term HPV persistence in young WLWH and HIV[-] women living in a SSA setting.

IV. SIGNIFICANCE

The proposed study will provide the much-needed evidence regarding the long-term protection afforded by HPV vaccination in WLWH living in SSA, who are at the greatest risk of HPV-related cancers. To our knowledge, there are no studies or trials to measure the effectiveness of HPV vaccination in WLWH living in SSA who were vaccinated at the optimal ages, prior to

sexual initiation. Most trials have focused on late teens and early adult PLWH, many of whom have already been exposed to HPV, and those trials have been conducted in high-income countries so the generalizability to low- and middle-income countries, and specifically SSA, is unclear. IARC/WHO is conducting a study of Gardasil® impact in Rwanda and Bhutan but that study is not specifically targeting WLWH, and therefore will have very few WLWH (67, 75, 76). Rwanda is one of the few places in the world, and the only place in SSA and low- and middle-income countries, that the long-term effectiveness of HPV vaccination in WLWH can be examined.

Most studies of HPV vaccination impact focus on a single anatomic site and do not measure the multi-site exposure that is related to the total risk of HPV-related cancers. There is good but imperfect correlation of HPV infection at each anatomic site (77-80). Notably, the prevalence of anal HPV infection can be high in WLWH relative to HIV[-] women who are negative for that type in the cervix (77). Finally, this study will establish risk factors for early HPV type-specific persistence, a key step in the development of cervical precancer and cancer (16, 81, 82) in WLWH living in SSA.

V. RELATIONSHIPS, COLLABORATIONS AND ROLES

This study is continuing and leveraging the Einstein-Rwanda consortium and local capacity developed in a previous SSA Collaborative HIV and Cancer Consortia (U54) grant. The consortium has established capacity to conduct large epidemiological and clinical studies on HPV and anogenital cancer, colposcopy, and treatment of high-grade cervical abnormalities, high-resolution anoscopy (HRA) and treatment of high-grade anal abnormalities, and laboratory assays including the AmpFire assay and DNA extraction, all of which will figure prominently in the proposed study. Drs. Gad Murenzi (Co-I; Research for Developmet (RD Rwanda) & Rwanda Military Hospital [RMH]) and Philip Castle (Co-I; National Cancer Institute [NCI]) again will co-lead the cervical study as they did for the successful cervical project in the previous U54-funded Einstein-Rwanda consortium. Dr. Anastos (PD/MPI; Einstein), PI of the Women's Interagency HIV Study and the Central Africa International Epidemiology Databases to Evaluate AIDS, will provide leadership and expertise on conducting clinical and epidemiologic studies. Drs. Leon Mutesa (MPI; University of Rwanda [UR]), Brad Aouizerat (co-I; New York University), and Robert Burk (co-I; Einstein) will develop the local capacity for studies of the human microbiome. Drs. Anastos and Burk have collaborated for over 20 years in the Women's Interagency HIV Study, with 40 shared publications. Drs. Castle and Anastos have worked together for 15 years to conduct studies of HPV in Rwandan women (83-86). Drs. Castle and Burk have collaborated for 20 years on molecular epidemiology studies of HPV, resulting in almost 50 publications together. Drs. Anastos and Aouizerat have collaborated for nearly 15 years in studies WLWH in the United States.

VI. APPROACH

VI.A. Study Design

This prospective observational HPV natural history study will assess the long-term effects of Gardasil® on HPV6/11/16/18 infection among WLWH. At local health clinics in Kigali, 3,028 women aged 18-28 years will be enrolled: 757 WLWH and 757 HIV[-] women who did receive HPV vaccination (birth cohorts 1996 and later) and 757 WLWH and 757 HIV[-] women who did not receive HPV vaccination (birth cohorts before 1996). The visual summary of the study is provided in **Appendix I**. At the enrollment visit, cervicovaginal, anal, and oral specimens will be collected and tested for HPV6/11/16/18 plus high risk HPV31/33/35/39/45/51/52/53/56/58/59/66/68 for exploratory aims. Participants positive for high-risk HPV and/or HPV6/11 on any sample will have a 6-12 month follow-up visit to measure HPV type-specific persistence - a surrogate endpoint recommended by the WHO (87). At young ages, the risk of precancer is very low, but for safety purposes, participants will be offered colposcopy if they have a persistent high-risk HPV cervical infection and anoscopy if they have a persistent HPV16/18 anal infection. Main outcomes will be prevalence and 6-12 month type-specific persistence of cervicovaginal (as an excellent proxy for cervical sampling (87)), anal, and/or oral infections by HPV6/11/16/18 as well as antibody titers for HPV6/11/16/18. Cervical and anal biopsy specimens diagnosed as CIN2+ or AIN2+, respectively, will also be tested for the 15 high-risk and 2 low-risk HPV types for secondary outcomes. In an exploratory aim, we will examine risk factors, including the cervicovaginal microbiome (88), for HPV type-specific persistence in WLWH and HIV[-] women.

At enrollment, blood will be collected for plasma from all participants. In the study laboratory, a stratified random sample of specimens will be selected to measure by ELISA anti-HPV16 and -HPV18 antibody titers and compare HPV-vaccinated WLWH. We will test titers in at least 1,302 samples (n≥548, 50% with three doses and 50% with two doses of Gardasil®, for both HPV-vaccinated WLWH and for HPV-vaccinated HIV[-] women; n≥108 for both HPV-vaccinated WLWH and for HPV-vaccinated HIV[-] women as controls) and up to 3,028 samples.

VI.B. Study Locations

In Kigali, the study staff will work with five public clinics that participate in the International Epidemiology Databases to Evaluate AIDS (IeDEA) program and the WE-ACTx private clinic (**Table 2**). Study investigators and staff successfully worked with these clinics in a cervical cancer screening study of 5,000 WLWH (83). If needed, the staff will work with up to four other public health clinics to recruit additional participants.

Table 2. Study enrollment sites	
IeDEA Sites	Non-IeDEA sites
1. Busanza health center (HC)	1. Cor-unum HC

2. Gikondo HC 3. Kicukiro HC 4. Nyarugunga HC 5. Rwanda Military Hospital 6. WE-ACTx private clinic	2. Kacyiru HC 3. Remera HC 4. Rwampara HC

VI.C. Target Population

This study will recruit women attending collaborating clinics and women from the communities surrounding those clinics. Three groups will be recruited:

- 757 HPV-vaccinated WLWH (Group 1),
- 757 HPV-unvaccinated WLWH (Group 2), and
- 757 HIV[-] women who are HPV-vaccinated (Group 3)
- 757 HIV[-] women who are HPV-unvaccinated (Group 4)

Within the HPV-vaccinated groups 1 and 3, the study will recruit at least 274 women with three doses and 274 women with two doses, with the remaining 209 will not be selected by the number of doses and may have 2 or 3 doses. The number of doses of HPV vaccination a woman received is dictated by introduction of HPV vaccination in Rwanda as previously shown in **Table 1**. In general, most Rwandan women born in 2003 or later will have been vaccinated with two doses of Gardasil®, women born between 1996 and 2002 will have been vaccinated with three doses of Gardasil®, and women born in 1995 or earlier will be unvaccinated.

Table 3. Target population by HIV status and age

HIV Status	**Approximate number of HPV vaccine doses**	**Birth year**	**Age at year of study enrollment (years)**		**N**
			in 2021	in 2023	
WLWH	3 doses	1996-2002	19-25	21-26	274
	2 doses	2003-2005	16-18*	18-20	274
	2 or 3 doses	1996-2005	19-25	18-26	209
	0 doses - unvaccinated	1993-1995	26-30*	28-31*	757
HIV[-]	3 doses	1996-2002	19-25	21-26	274
	2 doses	2003-2005	16-18*	18-20	274
	2 or 3 doses	1996-2005	19-25	18-26	209
	0 doses- unvaccinated	1993-1995	26-30*	28-31*	757
TOTAL					3,028

*Only women age 18-28 are eligible for enrollment.

Therefore, we will recruit by age, which will serve as a useful and very good proxy for HPV vaccination status, which otherwise would be difficult to confirm in real time at enrollment. HPV vaccination status then will be confirmed by retrospective review of the HPV vaccination records kept by the Rwanda Ministry of Health (MoH), who are collaborating on this project. The number of women recruited will therefore be stratified by age (as proxy for vaccine status) and HIV status (**Table 3**).

VI.D. Eligibility Criteria

VI.D.1 Inclusion Criteria

1. Gender: Female
2. Age: 18-28 years. The age of inclusion criteria will likely be restricted as age-specific enrollment goals are met.
3. Physically and mentally able and willing to participate in the study.
4. Willing to provide written and signed or thumb printed, informed consent.
5. Known to be living with HIV (i.e., enrolled in a treatment program), or consent to HIV testing to confirm HIV status.

VI.D.2. Exclusion Criteria

1. Have positive pregnancy test or report to be pregnant at the time of visit or less than 6 weeks post-partum (will be asked to make an appointment 6 or more weeks post-partum)
2. Report to be menstruating at the time of visit (will be asked to make new appointment)
3. History of hysterectomy and no longer have a cervix
4. History of treatment for cervical abnormalities after cervical screening
5. History of cervical cancer
6. Report no previous sexual activity
7. Because this study has age-specific enrollment goals for WLWH and HIV[-] women, once those enrollment goals are met for each study group, the respective cohorts will be closed and other eligible women will be excluded.

VI.E. Recruitment and Eligibility Screener

During the period of recruitment at a health clinic, WLWH and HIV[-] women will be solicited using advertisements throughout the entire clinic as well as outreach using community health workers (**Figure 1**). In addition, WLWH who are attending routine appointments at the HIV clinics will learn about the study during the group study overview sessions held in the health education room. All women who are interested in the study will meet with a study nurse who will give them an informational flyer and provide a brief verbal description of the study. The

study nurse will help the woman complete the eligibility screener that includes criteria listed above. If eligible and interested, the woman will register for an enrollment visit.

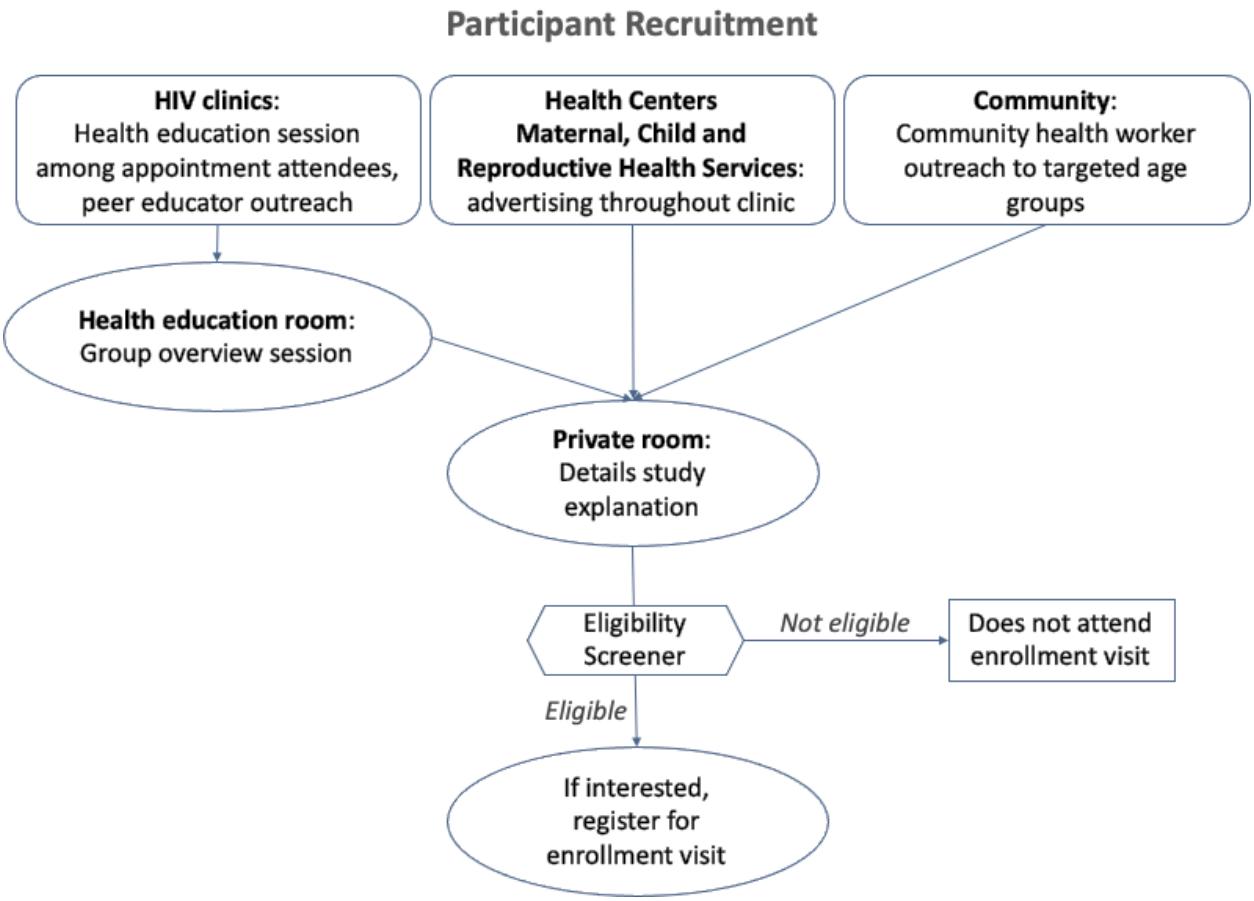


Figure 1. Participant recruitment

VI.F. Study Visits

VI.F.1. Enrollment Visit

VI.F.1.a. Informed Consent, HIV testing and Study Questionnaire

As outlined in **Figure 2**, at the enrollment visit conducted in a private exam room, the study nurse will describe the details of the study verbally and women will be provided an opportunity to ask any questions. The participant eligibility will be verified using the eligibility form. Women who report menstruating at the time of visit will be asked to make a new appointment 2 weeks later. Women who report to be pregnant or less than 6 weeks post-partum will be asked to make an appointment 6 or more weeks post-partum.

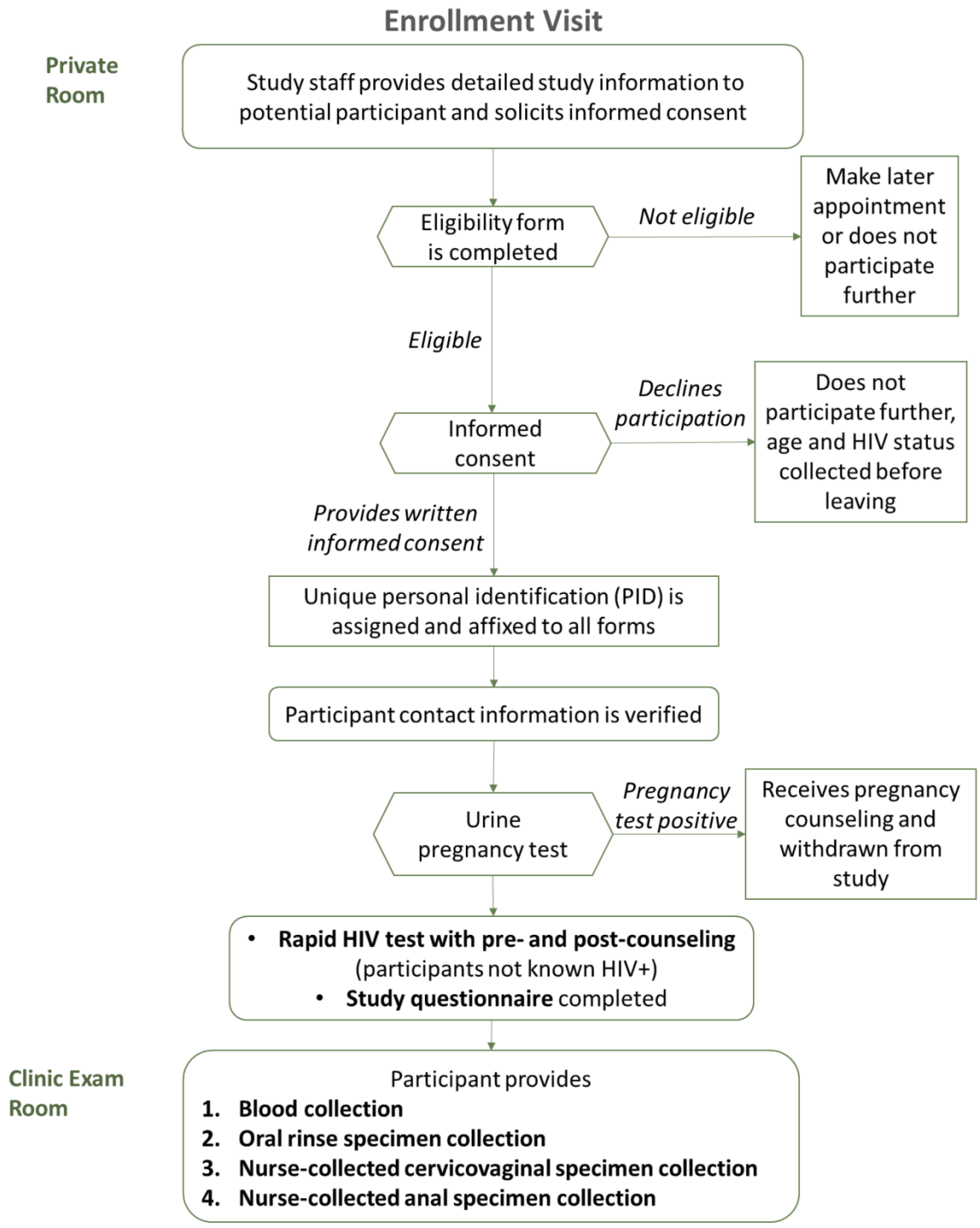


Figure 2. Enrollment visit procedures

Women will individually undergo the consenting process as described in **Section X.B.1.** Once the Informed Consent Form (**Appendix II**) is completed, a unique study participant

identification number (PID) will be assigned to the participant that will be used to identify all forms and specimens collected at the enrollment and follow-up visits.

Participants' personal contact information will be confirmed. Participants will provide a urine sample for pregnancy testing to confirm they are not pregnant. Counseling will be provided pre and post testing as needed. Participants who are not known to be living with HIV will have a rapid HIV test with pre- and post-HIV-testing counseling following the National Rapid HIV Diagnostic Testing Protocol. Any participant who tests positive will be referred to the health center's HIV clinic for HIV management following the Ministry of Health's HIV management guidelines. The consent form obtains the participants' permission to extract from medical records information related to their HIV care including CD4 count, viral load, ART use and age of initiation of HIV care. They will then complete a short questionnaire on socio-demographic information and risk factors for HPV including sexual behaviors (**Appendix III**). The questionnaire will be conducted in private, using an automated audio interview assistance tool Audio Computer-Assisted Self-Interview (ACASI) software. The study nurse will provide additional assistance only if requested.

VI.F.1.b. Blood Collection

For the study of anti-HPV16 and HPV18 antibody titers between WLWH and HIV[-] women (Specific Aim 2 listed above), the study nurse will collect one 5 mL tube of blood from all participants for HPV antibody serology testing.

VI.F.1.c. Cervicovaginal, Anal, and Oral Specimens

For the oral specimen, the nurse will hand a medicine cup with the saline rinse to the participant. The nurse will start the timer and instruct the participant to gargle, swish, and spit. For 30 seconds total, the participant will alternate between a 5-second swish and a 5-second gargle. The study nurse will help the subject count out the alternating 5 second squish and 5 second gargles at least 3 times. At the end of the 30 seconds, the study nurse opens the 5 oz. sterile specimen container and hands it to the participant, holding the top lid down to avoid contaminating it. The participant will spit the saline into the specimen container when done gargling. The study nurse will seal and label the specimen container with the participant ID.

Vaginal specimens will be collected using the AmpFire specimen collection brush (Atila BioSystems, Mountain View, CA, USA). The participant will lie on the exam table. The study nurse will insert the brush into the posterior vagina, turning the brush 3 turns left and right, before removing the brush. The study nurse will insert the brush into the collection tube and snap off the handle to break it. The study nurse will seal the tube and label it with the corresponding participant label.

Anal canal specimens will be collected using a scored Dacron swab. While the participant is lying on the exam table, the study nurse will collect the specimen by inserting a water-moistened, scored Dacron swab into the anal canal, turning the swab 2-3 turns left and right, before removing the swab. The study nurse will insert the swab into the collection tube and snap off the handle at the score on the handle to break it. The study nurse will seal the tube and label it with the corresponding participant label.

VI.F.1.d. Biospecimen Transport and Storage

A study staff will transport the oral saline rinse, dry swabs, and blood samples to the study lab. Blood samples will be processed the same day they arrive at the lab. If same-day processing is not possible, whole blood will be stored at 4–8°C for up to 24 hours before the serum/plasma is separated. Aliquots of plasma will be stored at -80°C freezer for further testing. Serum will be discarded.

Oral specimens will be processed and tested for HPV genotypes. Upon arrival, samples will be stored at 4°C until processed. Aliquots will be stored frozen at -20°C for further testing. After testing, residual specimens will be discarded.

Dry anal and cervicovaginal specimens will be processed and tested using the Atila Ampfire HPV genotyping. Upon arrival, the oral rinse specimen containers will be stored at 4°C refrigerators for processing the next working lab day. Dry brushes and associated labels will be stored in a box in the freezer at -20ºC for processing the next working lab day. After testing, residual specimens will be aliquoted and stored frozen at -20°C for further testing.

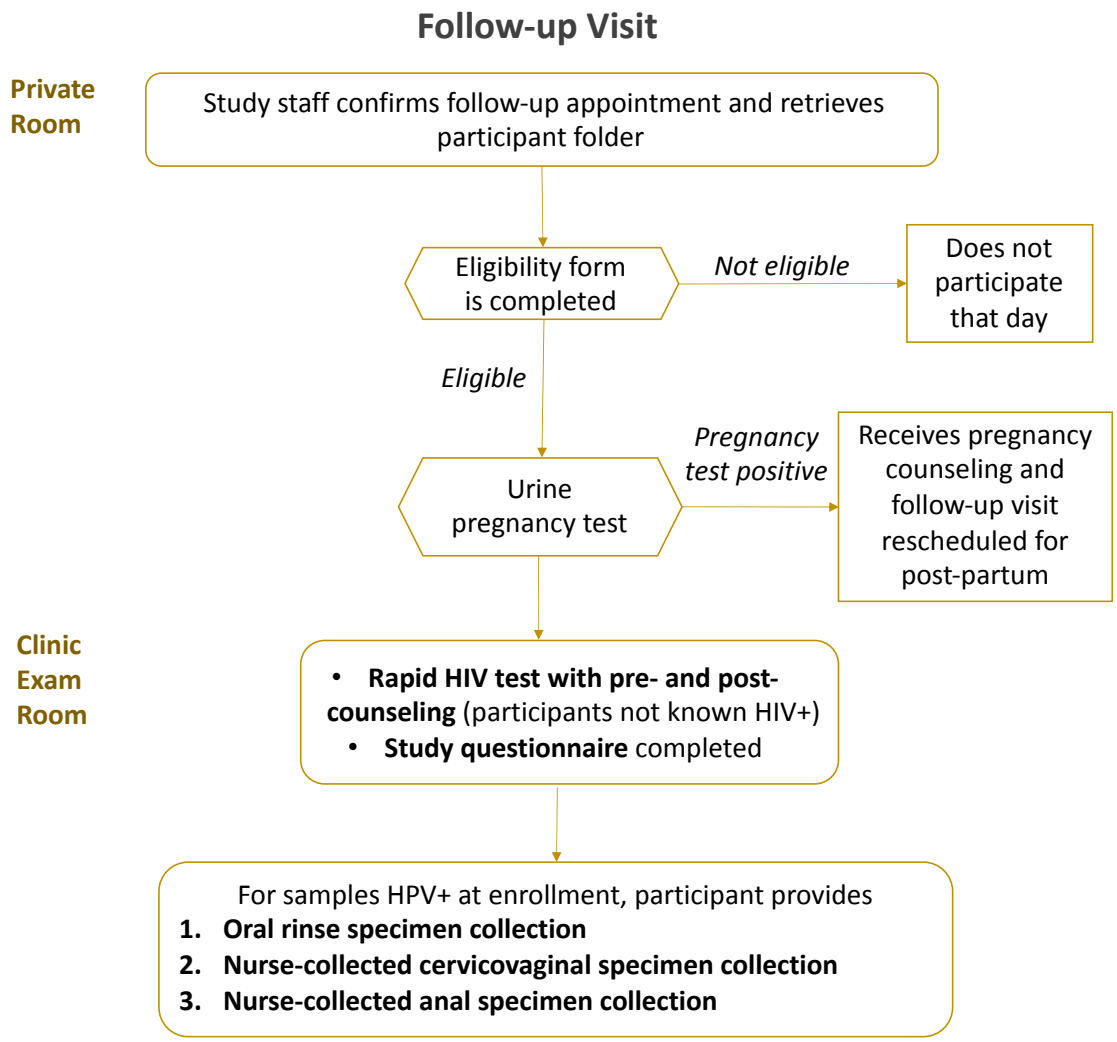
HPV genotyping will be conducted within 2 months of collection to allow ample time to schedule the 6-12 month follow up visit for women who test HPV positive for any HPV type at any tissue site.

VI.F.2. Follow-Up Visit of the HPV-Positive Cohort

Participants who test positive for any of the 17 HPV types on their cervicovaginal, oral, and/or anal specimen collected at baseline will be scheduled for a 6-12 month follow-up visit with a window of 5-15 months after their baseline visit; participants who test negative on all three specimens will exit the study. Objectives of the follow-up visit are to 1) measure 6-12 month HPV persistence, an accepted primary endpoint in prophylactic HPV vaccine trials (89), and 2) ensure the safety of the participants. As outlined in **Figure 3**, at the follow-up visit, the participant's eligibility status will be confirmed. Participants will provide a urine sample for pregnancy testing to confirm they are not pregnant. Counseling will be provided pre and post testing as needed. Participants unknown to be living with HIV will have a rapid HIV test with pre- and post-HIV-testing counseling following the National Rapid HIV Diagnostic Testing Protocol. Any participant who tests positive will be referred to the health center's HIV clinic for

HIV management following the Ministry of Health's HIV management guidelines. Only specimens from those anatomic sites that were HPV positive at baseline will be collected utilizing the same protocol as at enrollment. A study staff will transport the oral saline rinse and dry swabs to the study lab to be stored until study testing for 17 HPV genotypes; the follow-up cervicovaginal and anal specimens will be tested within one month to ensure timely follow-up of high-risk individuals who need diagnostic services (colposcopy and/or anoscopy).

Figure 3. Activities at follow-up visit



VI.G. Study Test Results and Clinical Management

VI.G.1. Management of Persistent Cervicovaginal HPV

A summary of the management of HPV-positive results is shown in **Figure 4**. To ensure the safety of participants, those who at the follow up visit, have a type-specific persistent HPV infection for one or more of the 15 hrHPV types (HPV16/18/31/33/35/39/45/51/52/53/56/58/59/66/68), regardless of HIV status, will be referred to colposcopy. Colposcopy and treatment will be performed at RMH by a trained study physician or gynecologist on staff at the hospital.

Digital images of the cervix will be taken using a study-provided contemporary digital camera (e.g., Samsung A21S or similar cellular phone camera). The participant's images will be shown to her if she so desires. All images from a participant will be identified using a study ID. Images will not be analyzed at the clinic visit but be stored in the study secure NIH server immediately or, if not possible at the time, images will be uploaded when secure access to the study server becomes available. Images will be used for later analyses with automated visual evaluation algorithms being developed at the NCI for management of HPV-positive women (90).

Biopsies will be taken of all acetowhite, visible cervical abnormalities consistent with the American Society for Colposcopy and Cervical Pathology (ASCCP) management guidelines (91). The colposcopy impression and location of biopsies will be recorded for clinical management files and saved in the participant record for study purposes.

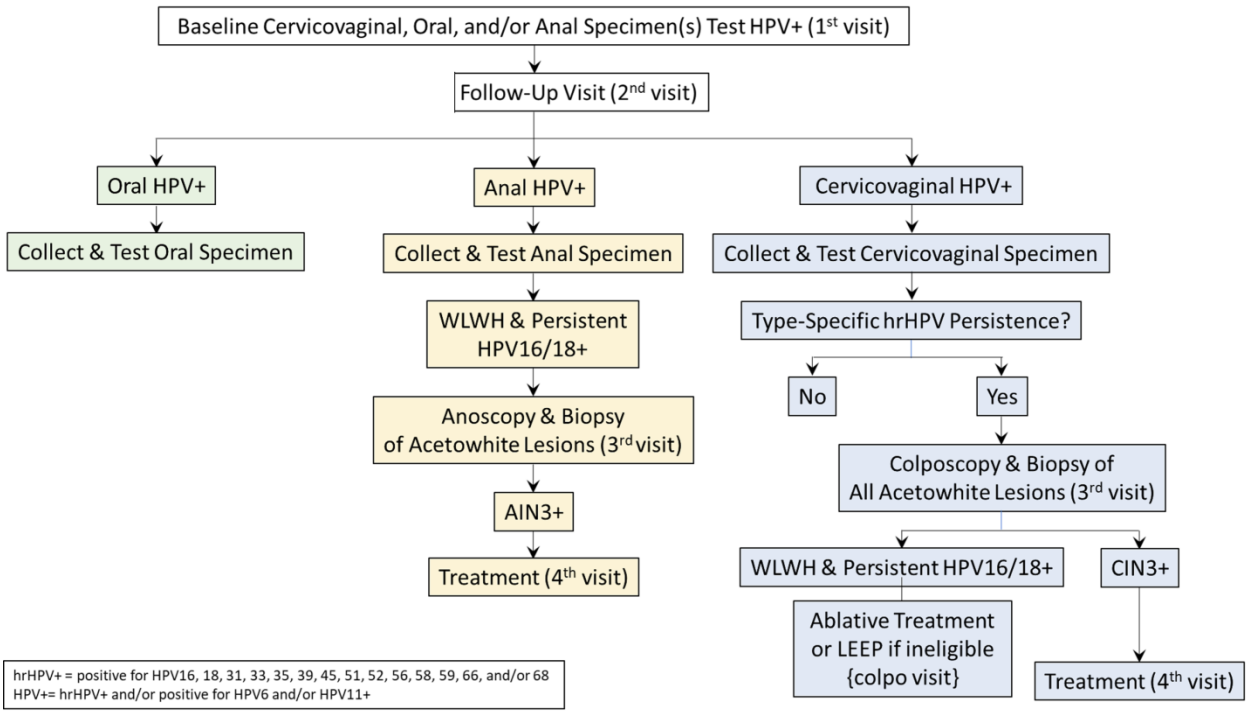


Figure 4. Follow-up and Clinical Management of Baseline HPV-Positive Results

After undergoing colposcopy and biopsies, WLWH with persistent HPV16- or HPV18 infection will receive ablative treatment unless ineligible for ablative treatment per WHO Guidelines (92); those ineligible for ablation will receive treatment by LEEP. Biopsies will be read by two local pathologists and Dr. Hébert (Einstein). All participants diagnosed with CIN3+ will undergo treatment. In accordance with ASCCP guidelines, those diagnosed with CIN2 will not undergo immediate treatment due to the low risk of ICC following a CIN2 diagnosis, especially in young women, and the possible increased risk in negative reproductive outcomes such as pre-term delivery following treatment (10). They will be advised to seek clinical follow-up in a year.

After processing and reading according to local practices, formalin-fixed paraffin-embedded (FFPE) tissue specimens from biopsies and excisional treatment with results of CIN2+ will be sent to the study lab for HPV typing for study purposes **(Section VI.I.1.)**.

To summarize, within the context of the research protocol, participants at risk of precancer will be provided with colposcopy as well as treatment and follow-up. Participants with a diagnosis that requires treatment beyond ablation or excision (i.e., invasive cervical cancer), will be referred and guided to appropriate local care.

VI.G.2. Management of Persistent Anal HPV

WLWH with persistent HPV16 or HPV18 infection will undergo anoscopy; HIV[-] participants with persistent HPV16- or HPV18 infection will not undergo anoscopy due to their still low absolute risk of anal cancer (10) and the morbidity of treating anal abnormalities. Anoscopy and treatment will be performed at RMH by a study physician on staff at the hospital with training for this protocol.

A biopsy will be taken from acetowhite lesions. The anoscopy impression and location of biopsies will be recorded for clinical management files and saved in the participant record for study purposes. After processing and reading according to local practices, formalin fixed, paraffin embedded (FFPE) tissue specimens from biopsies and excisional treatment with results of anal intraepithelial neoplasia (AIN) grade 2 (AIN2) or more severe diagnoses (AIN2+) will be sent to the study lab for HPV typing analysis **(Section VI.I.1.)**.

Those with a biopsy diagnosis of AIN grade 3 (AIN3) or anal cancer will be treated or referred for anal cancer management, respectively. Those diagnosed with AIN2 will not undergo immediate treatment due to the low risk of anal cancer following a AIN2 diagnosis and the aforementioned morbidity associated with treatment. Those with untreated AIN2 or persistent anal hrHPV by non-HPV16/18 types will be advised to seek clinical follow-up in a year.

Similar to colposcopy, within the context of the research protocol, participants at high risk of anal precancer will be provided with anoscopy as well as treatment and follow-up. Participants with a diagnosis that requires treatment beyond routine care (i.e., invasive anal cancer) will be referred and guided to appropriate local care.

VI.G.3. Management of Persistent Oral HPV

There are no management guidelines or evidence-based intervention for type-specific persistent HPV. Therefore, participants will not receive any clinical intervention for type-specific persistent oral HPV.

VI.G.4. Study Exit

Other participants, including WLWH and/or those with persistent low-risk HPV6- or HPV11 infection, will exit the study.

VI.H. Biospecimen Processing, Storage, and Shipment

The study lab will process, store and test for HPV in oral specimens in saline solution, dry samples, blood samples and FFPE tissue specimens. All specimens will be tested for individual 17 HPV types using the AmpFire HPV genotyping test.

In preparation for testing, oral specimens will be aliquoted, the dry sample will be rinsed into a vial with lysis buffer and FFPE tissue specimens will be suspended in lysis buffer. Specimens will be processed according to manufacturer's instructions. All residual lysed specimens will be stored at the study lab at -80^{o}C for later testing. Select samples will be sent to study collaborators' laboratory for microbiome testing. Additional microbiome testing will be conducted at the study lab.

Whole blood specimens will be processed to obtain aliquots of plasma. Following the protocol established by the Frederick National Laboratory of Cancer Research (FNLCR) HPV Serology Laboratory, select plasma specimens will be sent to FNLCR for testing and others will be tested at the study lab **(Section V.I.2).** After ELISA testing, residual plasma will also be stored at the study lab for later testing.

A biospecimen bank containing residual material from samples collected will be established at the study lab for subsequent methodologic and etiologic research.

VI.I. Laboratory Testing

VI.I.1. AmpFire HPV Testing

For HPV genotyping of cervicovaginal, anal, and oral specimens for Specific Aim 1 (**Section VII.A**), we will use the AmpFire HPV genotyping test (Genotyping High Risk HPV Real Time Fluorescent Detection; Atila Biosystems, Mountain View, CA, USA), which uses real-time PCR to detect 15 individual HPV genotypes HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68, plus low-risk HPV6 and 11, in 5 assay reactions previously established at the study lab and validated against other commercially available assays at RMH (93-96). This method requires no transport buffer (dry specimens) and no DNA purification step and is well suited to be run in clinic settings by personnel with relatively minimal training. For oral specimens in saline solution, specimens will first be spun in the centrifuge to enrich the sample. The AmpFire system provides results for each type within 1.5 hours. Atila has agreed to provide a separate detection tube for the combined detection of HPV6 and 11 (HPV6/11) for this study. The assay will be run per the manufacturer's instructions. The residual lysed specimen will be neutralized according to the manufacturer's protocol using its buffer and stored at -20^{o}C for long term storage and later testing.

FFPE tissues diagnosed as CIN2+ or AIN2+ will similarly have HPV genotyping using the AmpFire system according to the manufacturer's protocol.

For monitoring of quality of Ampfire testing, aliquots of selected samples will be sent to an independent lab for testing with a validated HPV typing assay. The results will be compared with local tests results for quality control and not clinical management. In some cases of negative Ampfire and the positive QC test, participants be referred for follow-up visit. Treatment will not be determined by the QC result.

VI.I.2. HPV Antibody Titers

For Specific Aim 2 (**Section VII.B**) anti-HPV16 and HPV18 IgG antibody titers will be measured from plasma by an ELISA based on a previously described method (97, 98) and according to the protocol established by the FNLCR HPV Serology Laboratory (99). Only anti-HPV16 and HPV18 IgG antibody titers, and not titers for all 4 HPV types, will be evaluated as antibody titers are correlated between antibody responses for the different types. HPV16 and HPV18 are the two most important HPV types and cause approximately 70% of cervical cancers (19). HPV16 tends to induce the highest titers while HPV18 tends to induce the lowest titers among the 4 types (100, 101) and therefore measuring the two types gives us a range in responses while using study resources cost effectively. The FNLCR Serology Laboratory will teach the ELISA protocol to Dr. Johanna Daily (co-I; Einstein), who will oversee the transfer of this ELISA assay to the study lab in Rwanda.

First, 20% of each group will be run at the Frederick NCI lab, then the results will be replicated at the study lab (masked to the original results), and then the remaining 80% of the testing will be finished at the study lab. As an additional quality control measure, a 10% random sample of specimens will be re-run at the NCI Frederick lab (masked to the original results) (inter-laboratory reliability) and another 10% random sample of specimens at the study lab (intra-laboratory reliability). The study lab will achieve at least 90% correlation for both anti-HPV16 and -HPV18 titers with FNLCR Serology Laboratory on the first 20% of specimens tested before the study lab embarks on testing the last 80% of specimens (101). Positive (e.g., IS standards (102, 103)) and negative controls (e.g., negative plasma) will be included in some testing plates to monitor assay performance per WHO recommendations (104).

VI.I.3. CVM Analyses

Baseline and follow-up specimens collected for baseline HPV-positive participants will be tested for cervicovaginal microbiota by next-generation sequencing (NGS) for our Exploratory Aim (**Section VII.C.**) on short-term HPV persistence. The first 20% of specimens will be tested in both the collaborating lab and the study lab until acceptable agreement is achieved (see below) after which the remaining 80% of specimens will be tested at the study lab with retesting 10% for quality control.

VI.I.4. Pathology

All histopathology slides will be scanned at the study lab and reviewed by two local pathologists and the Einstein study pathologist (105). If the diagnoses are concordant, no further review of the case will be performed. If the diagnosis is discordant and at least one pathologist diagnoses CIN2+, the biopsy slide will be subjected to a joint review and consensus diagnosis. For Negative/cervical intraepithelial neoplasia grade 1 pairs of diagnoses, which does not influence our analyses or the care of the participants, there will be no joint review.

VII. STATISTICAL SECTION

VII.A. Specific Aim 1

To measure population effectiveness of prophylactic HPV vaccine in reducing cervicovaginal, anal, and/or oral prevalent and persistent infections by HPV6/11/16/18.

VII.A.1. Analyses.

We will calculate the point prevalence and the prevalence of persisting infection by individual HPV-type specific infections, with binomial 95% confidence intervals (95% CI), for each anatomic and for the woman (all anatomic sites) for Gardasil®-vaccinated WLWH, Gardasil®-vaccinated HIV[-] women, unvaccinated WLWH and unvaccinated HIV[-] women. We likewise will calculate the point prevalence and the prevalence of persisting infection with 95%CI for all

HPV types in aggregate and in sub-groups of HPV types according to the protection afforded by Gardasil®: Gardasil®-targeted types (HPV6, 11, 16, and 18), Gardasil®-untargeted types for which there might be cross-protection (HPV31, 33, and 45) (40, 106, 107), and Gardasil®-untargeted HPV types for which there is little or no evidence of cross-protection (HPV35, 39, 51, 52, 53, 56, 58, 59, 66, and 68). Results will be stratified by age and number of doses (3 vs. 2) as well as other factors possibly associated with HPV prevalence. Differences in the prevalence of these HPV type sub-groups (targeted, possible cross-protection, and untargeted) between the four study groups of women will be tested using Fisher's exact or Pearson chi-square test. Differences in the prevalence of these HPV type subgroups by age, number of doses (3 vs. 2), and other factors within the group, will be tested for statistical significance ($p<0.05$) using a Fisher's exact or Pearson chi-square test.

Notably, the study groups of participants are fundamentally different populations (vs. a randomized control trial that would recruit from the same population and, as result of randomization, enroll similar, representative populations in each arm). Specifically, there are known differences in age (and therefore possible differences sexual behaviors) between Gardasil®-vaccinated vs. -unvaccinated WLWH, and possible differences in sexual behaviors between Gardasil®-vaccinated WLWH vs. HIV[-] women (since HIV infection predominately is sexually transmitted in this population). Therefore, we will use a relative measure of effectiveness to account for the differences in age and possible differences in exposure to HPV. We will use logistic regression to calculate the odds ratio (OR) of the point prevalence and the prevalence of persisting HPV infections of Gardasil®-targeted HPV types, individually (HPV6, 11, 16, *or* 18) and in aggregate (HPV6, 11, 16, *and* 18), vs. untargeted HPV genotypes for which there is no evidence of cross-protection, for each anatomic site individually (cervix, anus, OR oral cavity) and combined (cervix, anus, AND oral cavity). That is, we will compare the ratio between study groups of participants as follows:

$$\frac{\mathbf{N}_{HPV6/11/16/18}/\mathbf{N}_{HPV35/39/51/52/53/56/58/59/66/68}\ \text{[vaccinated WLWH] (Group 1)}}{\mathbf{N}_{HPV6/11/16/18}/\mathbf{N}_{HPV35/39/51/52/53/56/58/59/66/68}\ \text{[unvaccinated WLWH] (Group 2)}}$$

or

$$\frac{\mathbf{N}_{HPV6/11/16/18}/\mathbf{N}_{HPV35/39/51/52/53/56/58/59/66/68}\ \text{[vaccinated WLWH] (Group 1)}}{\mathbf{N}_{HPV6/11/16/18}/\mathbf{N}_{HPV35/39/51/52/53/56/58/59/66/68}\ \text{[vaccinated HIV[-] women] (Group 3)}}$$

or

$$\frac{\mathbf{N}_{HPV6/11/16/18}/\mathbf{N}_{HPV35/39/51/52/53/56/58/59/66/68}\ \text{[vaccinated HIV[-] women] (Group 3)}}{\mathbf{N}_{HPV6/11/16/18}/\mathbf{N}_{HPV35/39/51/52/53/56/58/59/66/68}\ \text{[unvaccinated HIV[-] women] (Group 4)}}$$

This will allow us to account for differences in HPV exposure, prevalence, and persistence due to differences in age (n.b., prevalence equals incidence * duration; prevalent infections at older ages tend to be more persistent ((108)) and sexual behaviors between groups. Additional logistic regression models may be used to adjust specifically on other factors, including age, number of doses, and sexual behaviors, to account for population differences between study groups.

Similarly, exploratory analyses will compare the effectiveness of HPV vaccination for cross-protection types and endpoints of cumulative detection of Gardasil®-targeted HPV types-associated CIN2+ and/or anal intraepithelial neoplasia grade 2 or more severe (AIN2+) (vs. untargeted HPV type-associated CIN2+ and/or AIN2+) between study groups.

For participants in the existing IeDEA database we will extract HIV specific variables. For those who we cannot match to the IeDEA database, will extract HIV specific variables from medical records.

In another exploratory analysis, among WLWH, the presumed route of HIV acquisition (either maternal to child or behavioral) will be compared with HPV positivity and, among HPV-positive women, HPV viral load.

VII.A.2. Sample Size/Power for Gardasil®-vaccinated vs. unvaccinated WLWH.

For this analysis, we will make the following assumptions: 1) at least 30% prevalence of HPV infection (of any anatomic site) at baseline, 25% of which will be Gardasil®-target HPV types and 55% will be untargeted HPV types, 2) a 10% loss to follow-up (LTFU) in at follow up visit, and 3) at least 70% of HPV infections persist for 6-12 months We justify an HPV prevalence of at least 30% based on 30% prevalence of high-risk HPV infection of the cervix in 30-year-old WLWH from our previous U54 study (109). We expect that the HPV prevalence may be higher since the prevalence of HPV, like that of other sexually transmitted infections, tends to peak about 5 years after the median age of sexual initiation in a population. However, we conservatively used 30% to ensure adequate statistical power. We justify our assumption of a maximum of 10% follow-up, given our experience working in Rwanda over the last ~20 years. We justify 70% of prevalent HPV infections persisting for 6-12 months, given that 36-50% of prevalent HPV infections persist 6-months in HIV[-] women (109, 110) and WLWH have an impaired immunity to HPV (8, 9).

Sample size calculation relates to the relative effectiveness measure as discussed. Under above assumptions, a sample size of 757 vaccinated and 757 unvaccinated WLWH will provide ≥80% power (p=0.05) to detect an OR of ≤0.5 for 6-12 month persistent HPV6/11/16/18 infections, relative to HPV35/39/51/52/53/56/58/59/66/68 (but not HPV31/33/45 because of possible cross protection), in Gardasil®-vaccinated vs. -unvaccinated WLWH. Consequently, because there will be more participants with a prevalent infection than 6-12 month persistent HPV infection (because there are no losses to follow-up or HPV viral clearance for which to account), there will

be ≥80% power (p=0.05) to detect a OR of ≤0.75 in point prevalence of HPV6/11/16/18 infection relative to HPV35/39/51/52/53/56/58/59/66/68, in Gardasil®-vaccinated vs. -unvaccinated WLWH.

VII.B. Specific Aim 2

To quantify, and examine the determinants of, long-term antibody responses to, HPV vaccination.

VII.B.1. Analyses.

Geometric mean titers (GMT) and seropositivity (seroconversion) will be compared between 1) HPV-vaccinated WLWH and HPV-vaccinated HIV[-] women, overall and stratified on the number of vaccine doses, 2) HPV-vaccinated and unvaccinated WLWH, overall and by the number of doses received by the HPV-vaccinated WLWH, and 3) three vs. two doses for HPV-vaccinated WLWH and 4) HPV-vaccinated and unvaccinated HIV[-] women, overall and by the number of doses received by the HPV-vaccinated HIV[-] women.

Differences in GMT and seropositivity will be tested for statistical significance using the Mann-Whitney and Fisher's exact tests, respectively. ANOVA and logistic regression models will be used to adjust for/assess the association of other factors (e.g., age at vaccination, age at enrollment into the study, number of doses, HIV status [positive vs. negative and current antiretroviral therapy, CD4 counts, and HIV viral load] and history [e.g., CD4 nadir], and detection of HPV genotypes, etc.) with GMT and seropositivity, respectively.

Among vaccinated and unvaccinated HPV seropositive WLWH, the association between HPV antibody titer and presumed route of HIV acquisition (either maternal to child or behavioral) will be examined.

VII.B.2 Sample Size/Power to compare Gardasil®-vaccinated WLWH vs. Gardasil®-vaccinated HIV[-] women.

Using the variance of GMT for HPV antibodies from Einstein et al. (111, 112), a sample size of at least 274 will provide 80% power (p=0.05) to detect a 30% difference in GMT between HPV-vaccinated WLWH and HIV[-] women for a given number of doses. If there is no appreciable difference in the titers between those who got 3 or two doses, we can combine those groups with different doses. In that case, with a sample size of at least 548, there will be 80% power (p=0.05) to detect a 22% difference between HPV-vaccinated WLWH and HIV[-] women.

VII.C. Exploratory Aim: A Study of 6-12 Month Type-Specific HPV Persistence

Given the size of the cohort of WLWH (n=1,514 at baseline), the planned follow-up of the HPV-positive WLWH, and the laboratory assay capacities that are available and will be

developed at the study lab, we have unique opportunity to look at determinants of early, short-term HPV genotype-specific persistence/clearance in young women. Studies of HPV persistence in WLWH, especially in SSA, are limited in number and size (113-120). There is a growing body of evidence that an altered CVM (cervicovaginal dysbiosis) is associated with persistent HPV infection (86) but there are no published data on its role in HPV persistence in WLWH. There is evidence of greater cervicovaginal microbiotic diversity in WLWH compared to HIV[-] women (121) and a complex, possible inter-dependence of CVM and HPV natural history (122). Given the importance of short-term HPV persistence as a biomarker of cervical-cancer risk (123) as well as an endpoint for prophylactic HPV vaccine trials, this is a unique opportunity to understand its determinants.

Paired baseline and follow up specimens from participants whose baseline cervicovaginal specimen tests HPV positive will have their cervicovaginal microbiome characterized. We will examine factors such as baseline and history (if available) of HIV disease status (HIV viral load and CD4), the presence of the HPV genotype at other anatomic sites, CVM composition, and other risk factors (e.g., sexual behaviors) on whether HPV infection persists vs. clears. Contingency tables with Pearson chi-square or Fisher's exact tests (categorical variables) or Kruskal-Wallis test (continuous variables) will be used to identify factors potentially associated with HPV genotype persistence (vs. clearance) ($p<0.1$). Vaginal community state types (CSTs) will be assigned to specimens based on hierarchical clustering of the top 20 OTUs, in terms of sample sums across all samples within the cohort. Prior to clustering OTUs will be agglomerated at the species level. In cases where an OTU could not be resolved down to the species level, the next lowest assigned taxonomic label will be used. Clustering was performed using the ward D2 algorithm using Euclidian distances. We will develop a multivariate logistic regression model that incorporates generalized estimating equations (GEE) to calculate odds ratios and 95% confidence intervals as a measure of association of these factors with the likelihood of HPV genotype-specific persistence (vs. clearance). Factors identified in the univariate analyses will be added to the model using both forward and backward selection techniques until a final, parsimonious model have been achieved. Future studies will examine predictors, including anatomic site-specific microbiome, of anal and oral 6-12 month HPV persistence and the inter-relationship of 6-12 month HPV persistence between anatomic sites.

VIII. CAPACITY BUILDING AND TECHNOLOGY TRANSFER

This study is funded through a U54 grant from the U.S. National Institutes of Health (NIH). Several components of this study require capacity building that will have long-term benefits for future research in Rwanda.

1. Next Generation Sequencing (NGS) technology will be established at the study lab to perform cervicovaginal and oral microbiota characterization. This will support future investigations of the microbiome in sickness and health. A biobank of cervicovaginal and

anal specimens will be created to understand the role of anatomic site-specific microbiota on corresponding HPV natural history. More generally, with these capacities we can look at the impact of HIV infection on anatomic site-specific microbiota. In addition, the capacity for doing NGS will allow locally conducted studies of the human genome and genomic testing for personalized medicine in Rwanda.

2. This study builds upon current ELISA capabilities at the study lab to perform titration of plasma antibodies, a skill that can be applied to other studies of vaccine response.

3. The current vaccine records for participants living in Kigali will be migrated into a common electronic database, which will allow us to conduct studies more easily on the impact of HPV vaccination on outcomes. Importantly, this will allow linkage of HPV vaccination status to both the HIV registry that the Rwanda MoH has and the Rwanda Cancer Registry, which we re-established to investigate the long-term impact of HPV vaccination on cancer incidence in WLWH and HIV[-] women. Through collaborations with Rwanda MoH, the HIV registry (TRACnet) will be linked with the cancer registry.

IX. RECRUITMENT AND RETENTION

IX.A. Community Engagement

The recruitment process will include the community health workers and health center staff reaching out to the women, providing education, and registering for an enrollment visit. In addition, we have a multi-faceted strategy to engage the community before and during the study.

IX.B. Benefits to Participation

Participants who have not previously tested positive for HIV will be provided an opportunity for rapid HIV testing with pre- and post-counseling. Because this study is recruiting young women, it is very unlikely that they will have precancer, but by participating in this study, their precancer will be diagnosed and treated.

IX.C. Barriers to Participation

The main barrier to participation is transportation to the study site, for which all participants will be reimbursed. All study procedures and services will also be paid for by the study. This study will ask questions about women's sexual history and reproductive health that might make them feel uncomfortable. They have the right to not respond to any question with which they are uncomfortable.

IX.D. Informational Materials

All participant education and informational materials as well as consents and questionnaires will be culturally adapted and available in English and Kinyarwanda. We will engage with an Advisory board, community health workers, and clinical staff to prepare these materials. All questions will be administered in Kinyarwanda by an automated audio interview assistance tool to ensure privacy and overcome any literacy barriers.

IX.E. Planning and Timeline

We are recruiting 3,028 women over a 14- to-16-month recruitment period, with an expectation of retaining 90% within the first 6 months-12 months of the study, which defines the main study endpoint. In the beginning, we expect that enrollment will take one hour per woman but will decrease to 30-45 minutes per participant within a few months as the protocol becomes routine for the clinical staff. This has been our experience in doing research in Rwanda for 20 years. For a dedicated staff of at least 5 full-time nurses and data entry personnel, and 25% clinician time, we believe this to be very feasible.

We have planned for developing the SOPs, procurement, piloting, staff training, development of data collection instruments and systems, and tools for quality control. We are budgeting to staff the clinical team and reimbursement for travel of staff and participants for the entire study to ensure the timely completion of the study as well as the continuity in the conduct of the study and the care of participants.

IX.F. Recruitment Strategies

Potential participants will be recruited from local health clinics and surrounding communities. The study staff will work with five public HIV clinics that participate in the IeDEA program in Kigali and the WE-ACTx private HIV clinic. If needed, the staff will work with up to four other public health clinics to recruit additional participants. During the period of recruitment at a health clinic, WLWH and women who are HIV[-] will be solicited using advertisements throughout the entire clinic as well as outreach using community health workers. In addition, WLWH who are attending routine appointments at the HIV clinics will learn about the study during the group study overview sessions held in the health education room.

IX.G. Retention Strategies

We will rely on the study staff to retain the participants through skills learned from previous trainings and experiences. We will monitor enrollment and retention through weekly reports generated from Research Electronic Database Capture (REDCap).

IX.H. Diversity

The Rwandan staff, which has extensive experience working in this community, is very knowledgeable about the population. We will be able to work with and through RD Rwanda and RMH, the Advisory board, and the community health workers to ensure that a representative population of WLWH and the community surrounding the health clinic is recruited into the study. As part of the training for the study, we will make certain that the clinical team recruits without bias.

IX.I. Staff

The staff is already receiving the necessary training during our previous U54 grant (5U54CA190163) to conduct this study. However, we are allocating several months for protocol development and staff training before enrollment starts. The local staff will be responsible for providing most of the clinical services and increasingly so as they get additional training.

X.PROTECTION OF HUMAN SUBJECTS

X.A. Risks to Human Subjects

X.A.1. Human Subjects Involvement, Characteristics, and Design

This is an observational study to assess the long-term population effectiveness and immunity of the HPV vaccine (Gardasil®) in Rwandan WLWH. The study will compare the cervicovaginal, anal, and oral prevalent and 6-12 month persistent HPV6/11/16/18 infections in HPV-vaccinated WLWH to those in unvaccinated WLWH and HPV-vaccinated HIV[-] women. We will also compare the HPV immune response in HPV-vaccinated WLWH to HPV-vaccinated HIV[-] women and the impact of switching from 3 doses to 2 doses of Gardasil® in 2015. Finally, the study will investigate the risk factors, including the CVM, for HPV persistence in WLWH. The long-term goal is to establish a cohort of WLWH in whom we can examine the long-term effectiveness of HPV vaccination in WLWH now and in the future.

The study will include 757 WLWH and 757 HIV[-] women who did receive vaccination (birth cohorts 1996 and later) and 757 WLWH who did not receive HPV vaccination (birth cohorts before 1996) and 757 HIV[-] women who did not receive vaccination (birth cohorts before 1996). Women attending collaborating clinics and the surrounding communities will be recruited by community health care workers and study staff. All women who are interested in the study will meet with a study nurse who will give them an informational flyer and provide a brief verbal description of the study and complete the eligibility screener.

At the enrollment visit, the study nurse will describe the details of the study verbally and women will be provided an opportunity to answer any questions. To meet the eligibility criteria, women will need to confirm they are 18-28 years old, willing, and physically and mentally able to

participate, willing to provide written and signed or thumb printed informed consent, and willing to take an HIV test if they are not known to be living with HIV. Women who report to be menstruating, pregnant or less than 6 weeks post-partum will be asked to make a later appointment. Women will be excluded if they report no previous sexual activity, have a history of cervical cancer, treatment for cervical abnormalities after cervix screening, or hysterectomy and no longer have a cervix. Because this study has age-specific enrollment goals for WLWH and HIV[-] women, once those goals are met, the respective cohorts will be excluded.

All participants must sign and date the most current IRB approved version of the study specific informed consent (**Appendix II**) prior to participating in the study or having any study specific procedures. It will be the responsibility of Dr. Fabienne Shumbusho and the coordinator/study manager to ensure that participants are consented with the latest approved version of the informed consent. The study staff will ensure that all sections of the informed consent that require completion by participants are completed.

If a participant does not sign an informed consent prior to a study specific procedure, the participant will not be considered eligible and will not be screened or registered.

This study is continuing and leveraging the Einstein-Rwanda consortium and local capacity developed in a previous SSA Collaborative HIV and Cancer Consortia (U54) grant. The consortium has established capacity to conduct large epidemiological and clinical studies on HPV and anogenital cancer, colposcopy, and treatment of high-grade cervical abnormalities, high-resolution anoscopy (HRA) and treatment of high-grade anal abnormalities, and laboratory assays including the AmpFire assay and DNA extraction, all of which will figure prominently in the proposed study. Drs. Gad Murenzi and Fabienne Shumbusho (RD Rwanda and RMH) will oversee the study with the lead study nurse Athanase Munyaneza. The recruitment, enrollment and follow-up visits will be conducted in coordination with five public clinics that participate in the IeDEA program and the WE-ACTx private clinic. If needed, the staff will work with up to four other public health clinics to recruit additional participants. The research clinic at RMH will provide colposcopy and anoscopy examinations with treatment as well as referral for advanced lesions as needed.

X.A.2. Study Procedures, Materials, and Potential Risks

Most participants will have one study enrollment visit in a private room. Contact information will be collected and stored securely in the study database. Participants will provide permission to obtain clinical information pertaining to HIV monitoring and treatment as well as HPV vaccination history. The visit will consist of a study questionnaire that uses ACASI to ask about socio-demographic information and risk factors for HPV including reproductive history and sexual behaviors. Participants will have a urine pregnancy test to confirm they are not pregnant. Those who are not known to be living with HIV will have a rapid HIV test. A study nurse will provide pre- and post-counseling for both pregnancy and HIV tests. All participants will provide

a blood draw for anti-HPV16 and HPV18 antibody titer testing. All participants will provide a cervicovaginal, anal, and oral specimens for high-risk HPV and HPV6 and HPV11 testing. Specifically, participants will provide an oral rinse sample collection by gargling and swishing a saline solution for 30 seconds before spitting the rinse into a specimen container. The study nurse will help the participant count time and provide the collection. Then, while lying on the exam table, the study nurse will collect a specimen taken vaginally (without the use of a speculum) using a cervical brush. The study nurse will then collect a sample from the anal canal using a scored, Dacron swab. In addition, participants not known to be HIV-infected will have a rapid HIV test with pre- and post-counseling.

We anticipate that approximately 30% of participants will test positive for high-risk HPV and/or HPV6/11 on any sample and be invited for a 6-12 month follow-up visit. At that visit, participants will provide only specimens from those anatomic sites that were HPV-positive at baseline to measure 6-12 month HPV type-specific persistence. For safety purposes, WLWH and HIV[-] women with a cervicovaginal type-specific persistent hrHPV infection will be referred to colposcopy, and WLWH with an anal type-specific persistent HPV16/18 infection will be referred to anoscopy (**Section VI.G**). At the colposcopy and anoscopy procedure and follow-up visits, participants will be treated as needed.

All participant data collected will be entered or transferred to a secure REDCap database, with access to personal information restricted to the local staff. Study data will be stored in REDCap and maintained on password protected study computers behind the institution firewall. REDCap is a secure, web-based application designed to support data capture for research studies providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

Potential risks from these procedures include:

- *Eligibility Screening.* Potential participants will be asked to confirm their history of any sexual activity and that might make them feel uncomfortable. This question will be asked in a neutral manner in a private location.
- *Urine Pregnancy Test.* A pregnancy test can produce anxiety both before and after testing. Participants will be asked before testing whether they believe they might be pregnant. If they test positive, the study nurse will provide counseling as needed for the unexpected result.
- *Rapid HIV Testing*. HIV testing can produce anxiety both before and after testing.
- *Cervicovaginal Collection*. Collection of cervicovaginal specimens involves a modest risk of bleeding and is typically very limited when it occurs. Testing positive for any test may

cause psychological distress (anxiety). Excessive bleeding from cervicovaginal specimen collection is very rare.

- *Anal Collection*. Collection of anal specimens involves a modest risk of bleeding and is typically very limited when it occurs. Testing positive for any test may cause psychological distress (anxiety). Excessive bleeding from anal canal specimen collection is very rare.
- *Oral Collection*. Collection of oral specimens involves a modest risk of bleeding and is typically very limited when it occurs. Testing positive for any test may cause psychological distress (anxiety). Excessive bleeding from the collection of an oral specimen is very rare.
- *Blood Draw*. There is occasionally the possibility of discomfort, bleeding, or bruising from the blood draw.
- *Colposcopy, Biopsies and Digital Cervical Images*. Biopsies may induce vaginal bleeding and may incur pain, infection, and short-term psychological distress (anxiety). Excessive bleeding from a biopsy is rare. A diagnosis of CIN2 or more severe diagnoses may cause psychological distress (anxiety). A diagnosis of invasive cervical cancer may cause severe psychological distress.
- *Anoscopy and Biopsies*. Participants may feel a cramp of pinch during the biopsy. Many people do not feel the biopsy. They may notice a scant amount of bleeding with a bowel movement or wiping for a couple days. Most often there are not serious side effects from this procedure. Complications such as bleeding or infection are rare but can occur.
- *LEEP*. LEEP may induce vaginal bleeding and may incur pain, infection, and short-term psychological distress (anxiety). Excessive bleeding from a LEEP is rare.
- *85% trichloroacetic acid for small lesions or infrared coagulation (IRC) for larger lesions.* 85% trichloroacetic acid uncommonly may induce local discomfort and pain. For IRC, there can be mild to moderate pain or discomfort which can be treated with pain medication. Bleeding or discharge with bowel movements can occur for up to 2-3 weeks. Rare complications (< 1%) include infection in the anal area and severe bleeding.
- *Ablative Treatments*. Ablative treatments typically induce pain, cramping, and watery discharge. Ablative treatment may cause infection, malodorous discharge, and cervicitis. Fainting due to a vasovagal reaction from cryotherapy or thermal ablation is rare. Vaginal bleeding is rare. Cervical stenosis is rare.
- *Questionnaire.* Participants will be asked questions about sexual history and reproductive health that might make them feel uncomfortable. They will be conducted in private, using an automated audio interview assistance tool ACASI.
- There is also the risk of psycho-social stress that would occur if there was inadvertent disclosure of confidential medical or other personal information. All medical and personal information will be provided to participants in a private study room to ensure privacy.
- Women may seek alternative screening and treatment services such as they exist in Kigali or elsewhere if they do not wish to participate in the study.
- The study investigators and staff will work with the local MoH and clinic staff to ensure all study activities will be conducted in compliance with local COVID-19 pandemic

guidelines. Necessary precautions will be taken to mitigate the risk of COVID-19 transmission among study participants and staff. This includes cleaning all tablets and headphones used by study participants and staff in between visits. All study staff and participants will wear masks and utilize hand sanitizer regularly. All surfaces will be frequently cleaned with disinfectant. Whenever possible, windows will be left open for ventilation.

X.B. Adequacy of Protection Against Risks

X.B.1. Informed Consent and Assent

Women will be recruited during interactions with the community health workers and health center staff. During this outreach women will be provided education and allowed to register for an enrollment visit.

At the enrollment visit in the private exam room, the study nurse will describe the details of the study verbally and women will be provided an opportunity to ask any questions. The participant eligibility will be verified using the eligibility form. Those who qualify and are able and willing will then undergo the consenting process, in which the two copies of the consent form, one for the study documentation and one for the participant to take home for her records. The consent form includes study information such as contact information for study doctors in case of questions, concerns, or emergencies. The study nurse and the participant will go through a consent form, which will be read by the study nurse to those participants who are illiterate, allowing for sufficient time for questions and answers about the procedures and processes.

All participants must sign and date the most current IRB-approved version of the study specific informed consent prior to participating in the study or having any study specific procedures. It will be the responsibility of Dr. Fabienne Shumbusho and coordinator/study manager to ensure that participants are consented with the latest approved version of the informed consent. The study staff will ensure that all sections of the informed consent that require completion by participants are completed.

If a participant does not sign an informed consent prior to a study specific procedure, the participant will not be considered eligible and will not be screened or registered. A copy of the informed consent will be archived in the participant's research file. The informed consent process will be documented in the study log.

X.B.2. Protections Against Risk

Protection against the risk of inadvertent disclosure of confidential information is addressed by the standard procedures at the Rwandan study site, including: (i) storing completed paper copies of study forms and other hard copy information (described above), identified by study number

only, in a filing system separate from the name-address file of participants in the study; and (ii) only the designated local personnel have access to cross-reference the files.

Copies of signed consents will be stored in locked file cabinets in a locked room, with access restricted to study personnel only, at the study lab. Data will be entered or transferred to a secure REDCap database, with access to personal information restricted to the local staff.

Study data will be stored in REDCap and maintained on password protected study computers behind the institution firewall. REDCap is a secure, web-based application designed to support data capture for research studies providing: 1), an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. REDCap is hosted on a secure server and has undergone a Governance Risk & Compliance Assessment by All REDCap electronic data files shared with Albert Einstein College of Medicine will be maintained by the HIPAA-compliant Epidemiology Study Management and Informatics Core Facility (ESMI) at Einstein. The Albert Einstein College of Medicine policy on use of REDCap can be found at: http://ric.einstein.yu.edu/ric_files/REDCap%20Appropriate%20Use%20Policy.pdf

Side effects/adverse events due to ablative treatments are generally minor (pain, cramping, and watery discharge). Any pain, bleeding, or stress that might occur related to colposcopy, biopsies, vaginal collections, and/or LEEP are typically modest and no greater than those risk for the primarily HIV[-] women in the general population for whom cervical screening is recommended by the WHO and most countries in the world. These side effects and serious adverse events will be managed through referral to the Gynecology department at RMH. As needed, consults with other medical specialists will be sought. Care for study-related adverse events will be paid for by the study. Serious adverse events will be reported to the IRBs.

Study nurses have been trained in pre- and post-counseling for HIV testing and will provide support to participants taking rapid HIV tests. They will be trained to assist participants with any anxiety or discomfort experienced through responses to questionnaires and/or testing HPV positive.

Although women who are pregnant are at very low risk of adverse events due to the enrollment and follow-up procedures in this study, it is important to avoid any potential perceived risk. For this reason, all participants will have a pregnancy test performed at all visits. If they are found to be pregnant, they will be exited from the study and invited to enroll when they are at least 6 weeks post-partum.

Risks associated with blood collections will be minimized by having a trained and experienced health worker draw blood.

Because of the nature of the study, we believe that there is a very low risk of incidental findings related to participants' health and well-being. That said, we will have language in the informed consent acknowledging the possibility of incidental findings, and whether and how that information will be disclosed confidentially to the individual. Should there be an incidental health finding, the information will be provided to the participant if they consented to do so, and the research team will ensure that basic educational information about the nature of the finding is made available and will assist in finding the appropriate referral to a clinician or specialist for care if so desired.

X.C. Potential Benefits of the Proposed Research to Research Participants and Others

Participants who have not previously tested positive for HIV will be provided an opportunity for rapid HIV testing with pre- and post-counseling. Any participant who tests positive for the rapid HIV test at enrollment will be connected to the local HIV clinic for evaluation and management. Because this study is recruiting young women, it is very unlikely that they will have precancer. But by participating in this study, their precancer will be diagnosed and treated. This study will generate important information contributing to the knowledge of HPV vaccine effectiveness and immunity in Rwanda women living with HIV and without HIV. The findings will help to inform the development of programs and interventions for communities.

XI. LIMITATION AND FUTURE DIRECTIONS

Several limitations in the proposed study are worth noting. Our study is not a randomized trial, but the study populations will be selected from different cohorts of women: WLWH who are too old to have been vaccinated, WLWH and HIV[-] women who were vaccinated with 3 doses, WLWH and HIV[-] women who were vaccinated with 2 doses, and HIV[-] women who were too old to have been vaccinated. We have proposed several approaches to account/adjust for those age differences but as with any observational study, these techniques are unable to completely control for this bias.

We are using 6-12 month persistence as a proxy for cancer risk but ideally, we want to measure the impact of Gardasil® on high-grade cervical and anal abnormalities as a more proximal surrogate for cancer risk. The population will be still too young to have large numbers of these endpoints although we will look at these endpoints in secondary analyses. The longer-term effectiveness of Gardasil® through years of peak HPV exposure cannot be assessed in the 5-year time period of a single grant.

Our hope is to conduct follow-up studies of this newly established cohort of WLWH to assess the durability of immunogenicity and protection, including protection against high-grade cervical and anal abnormalities, offered by HPV4 for WLWH, as well as continuing to study the natural history of HPV in WLWH living in SSA. Eventually, as noted above (**Section VIII**.), we will be

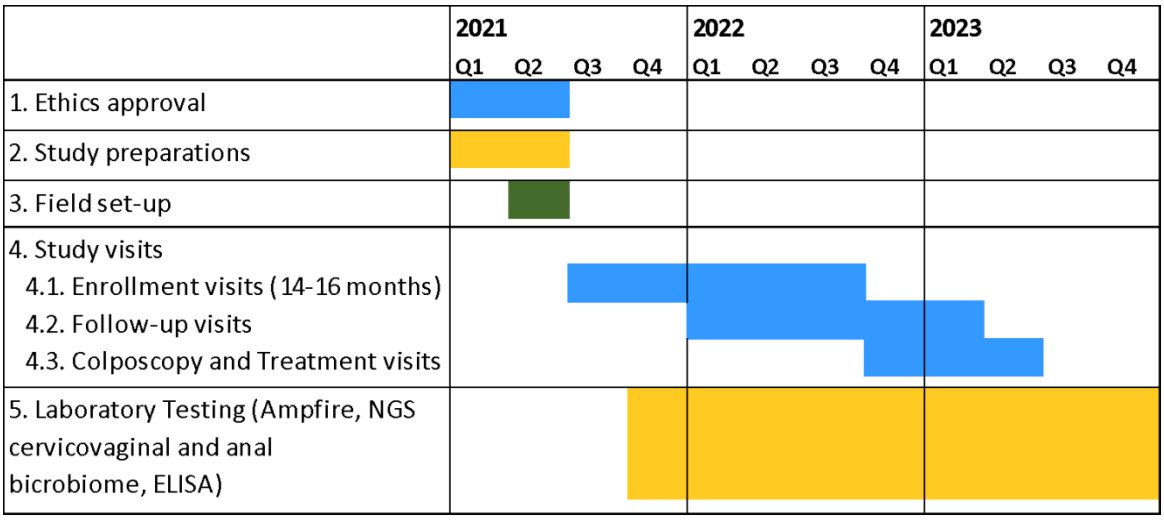
well-positioned to examine the impact of HPV vaccination on cancer incidence in WLWH by linkage of electronic databases that we have helped/will help create.

XII. DISSEMINATION OF RESEARCH RESULTS

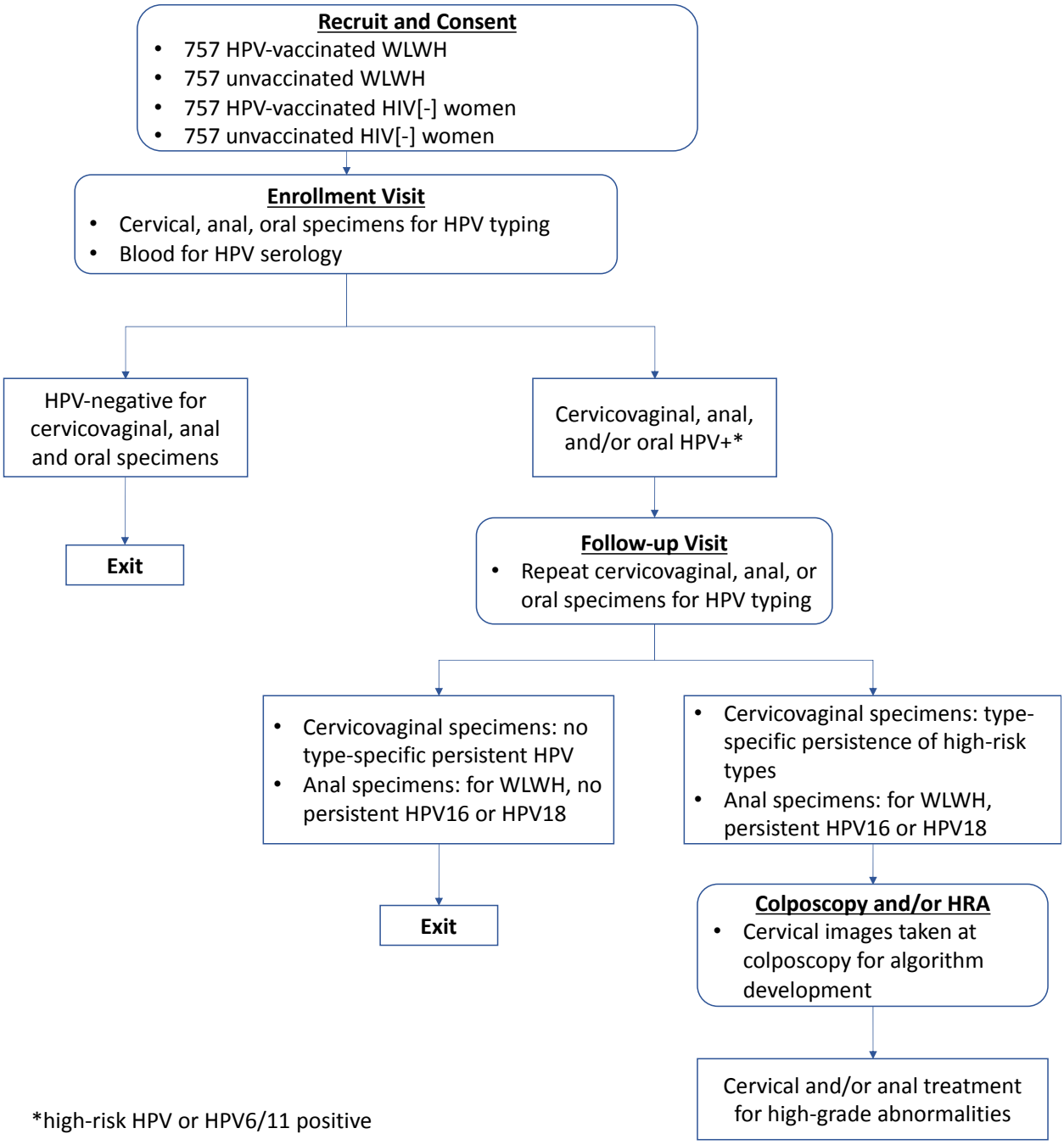
We plan to publish a series of 10 or more scientific reports in peer-review scientific journals. As building research capacity in Rwanda is a major goal of this U54 grant, all members of the research team will be asked and supported to lead at least one analysis and one manuscript preparation, based on interests and expertise. A publication grid is in development to assign analytic and publication responsibilities equally and collaboratively among both Rwandan and U.S. investigators.

XIII. TIMELINE

Pending approvals and situations in the field with the COVID-19 pandemic, we anticipate starting enrollment in September 2021. Enrollment will take approximately 14-16 months with completion of follow-up visits 6-12 months after that. All colposcopy and treatment visits should be completed by mid-2023.



APPENDIX I. VISUAL SUMMARY OF THE STUDY PROTOCOL



*high-risk HPV or HPV6/11 positive

APPENDIX II. WRITTEN INFORMED CONSENT DOCUMENT

KEY INFORMATION FOR LONG-TERM HPV VACCINATION EFFECTIVENESS AND IMMUNITY IN RWANDAN WOMEN LIVING WITH OR WITHOUT HIV

We are asking you to choose whether or not to volunteer for a research study about how well the HPV vaccine works for women living in Rwanda. This page is designed to give you key information to help you decide whether to participate. We have included detailed information

after this page. Ask the research team questions. If you have questions later, please contact the research investigator in charge of the study. Their contact information is below.

WHAT IS THE STUDY ABOUT AND HOW LONG WILL IT LAST?

Cancer of the cervix (the opening of the womb), anus, and throat can be caused by a virus – the human papillomavirus, also called HPV. We know that when the HPV vaccine is given to girls, it prevents infection from certain types of HPV. We want to understand how the vaccine prevents HPV infection for women many years after they were vaccinated. We also want to understand whether the vaccine works differently for women with HIV. By doing this study, we hope to learn among women like yourself in Rwanda, how well the vaccine works in the future. Your participation in this research will last about one year. Today, you will be asked to conduct a short questionnaire, provide a blood sample, and provide 3 samples of cells collected by a study nurse (vaginal, oral, and anal samples). If you do not have HIV, you will have an HIV test today. If any of these samples have HPV, we will ask you to return in 6-12 months for additional visits so you can provide samples again and see if you still have HPV or if it has gone away on its own. If you still have HPV, we may ask you to see a special doctor to make sure you do not have precancer.

REASONS YOU MIGHT CHOOSE TO VOLUNTEER FOR THIS STUDY

You will be adding to the knowledge of how HPV vaccination works in the future for women with different health conditions in Rwanda. Your participation will specifically help us understand how to improve HPV vaccination for women who live with HIV. For a complete description of benefits, refer to the Consent Document below.

REASONS YOU MIGHT CHOOSE NOT TO VOLUNTEER FOR THIS STUDY

We do not anticipate any mental, emotional, or physical harm through participation in this study. However, you may feel concerned about the confidentiality of the information shared as part of this research. Your responses will be kept completely confidential. None of the information you provide will be shared with anyone outside of the study team.

DO YOU HAVE TO TAKE PART IN THE STUDY?

Your participation is voluntary. You are being asked to complete the survey, but you may choose not to do so. You may choose to skip any questions that you do not wish to answer. If you sign the consent form document below, you have agreed to become part of the research study.

If you decide to take part, you are free to stop participating at any time without giving a reason. However, some of the information may have already been entered into the study and that will not be removed. The researchers may continue to use and share the information they have already collected.

WHAT IF YOU HAVE QUESTIONS, SUGGESTIONS OR CONCERNS?

The person in charge of the study is Gad Murenzi, MD, MPH. If you have questions, suggestions, or concerns regarding this study or you want to withdraw from the study. His contact information is:

Gad Murenzi MD, MPH, Principal Investigator
RD Rwanda, Kigali, Rwanda
Tel: +250 788 589 085 / Email: gadcollins@gmail.com

If you are unable to reach Dr. Murenzi, Dr. Fabienne Shumbusho, MD is another researcher responsible for this study that you can reach out to. Her contact information is:
Dr. Fabienne Shumbusho, MD
RD Rwanda, Kigali, Rwanda
Tel: +250 788 559 065 / Email: fabienneshumbusho@gmail.com

As the study will be conducted in Rwanda, if you wish to ask questions about the study or your rights as a research participant to someone other than the researchers or if you wish to voice any problems or concerns you may have about the study, please contact the chairperson of the Rwanda National Ethics Committee (RNEC) Dr. Mazarati Jean Baptiste at (250) 78830987 or Dr. Tumusiime David at (250) 788749398 or by mail: info@rnecrwanda.org.

If you have any additional questions, suggestions, or concerns about your rights as a volunteer in this research, contact staff in the Einstein Institutional Review Board (IRB) at irb@einstein.yu.edu.

RWANDA NATIONAL ETHICS COMMITTEE/ALBERT EINSTEIN COLLEGE OF MEDICINE
DOCUMENTATION OF INFORMED CONSENT AND HEALTH INSURANCE PORTABILITY AND ACCOUNTABILITY ACT OF 1996 (HIPAA) AUTHORIZATION

Introduction

You are being asked to participate in a research study called "**Long-Term HPV Vaccination Effectiveness and Immunity in Rwandan Women Living with and without HIV".** Your participation is voluntary -- it is up to you whether you would like to participate. It is fine to say "no" now or at any time after you have started the study. If you say "no," your decision will not affect any of your rights or benefits or your access to care.

Before you decide to be part of this research study, you need to understand the risks and benefits so that you can make an informed decision. This is known as informed consent. This consent form provides information about the study, which will also be explained to you. Once you understand the study and the risks involved, you will be asked to sign this form, or in the case that you are illiterate, provide your thumbprint on it and have it signed by a witness, if you want to take part. Remember, it is your decision: participation in the study is entirely voluntary.

If you cannot read, a study staff will help you read and understand the written paper that you will sign or thumbprint in the presence of a witness you trust, should you decide to join the study.

The researcher in charge of this project is called the "Principal Investigator." His name is **Gad Murenzi, MD, MPH**. You can reach Dr. **Murenzi** by phone at **(250) 788589085.** **Fabienne Shumbusho, MD** will be serving as a **Co-Investigator** of this study, and she can be reached by phone at **(250) 0788559065.** They are both researchers at: **Research for Development (RD Rwanda) and Rwanda Military Hospital** **Kigali, Rwanda** For questions about the research study, or if you believe you have an injury, contact the Principal Investigator, Co-Investigator, RNEC or the Einstein IRB.	The Rwanda National Ethics Committee (RNEC) and Institutional Review Board (IRB) of the Albert Einstein College of Medicine have approved this research study. The IRB # is in the stamp in the upper right-hand corner and bottom right corner as well. If you have questions regarding your rights as a research subject, you may contact RNEC chairpersons **Dr. Mazarati Jean** Baptiste at **(250) 78830987** or **Dr. Tumusiime David** at **(250) 788749398**. You can also reach the RNEC by email at info@rnecrwanda.org or by mail: **Rwanda National Ethics Committee** **Ministry of Health** **P.O.Box.84** **Kigali, Rwanda** If you have any additional questions, please feel free to contact the Einstein IRB at irb@einstein.yu.edu.
Support for this research study is provided by **the National Institutes of Health** (USA) through Grant No: 6U54CA190163-06.	

Why is this study being done?

HPV infection is responsible for most of cancer of the cervix (the opening of the womb), especially for those women with HIV infection. Some other less-common cancers found in the anus and the throat are also caused by HPV. For over 10 years, a vaccine for HPV has been given to older girls in Rwanda. We know that when the HPV vaccine is given to girls, it prevents infection from certain types of HPV. We want to understand how the vaccine prevents HPV infection for women many years after they were vaccinated. We also want to understand whether the vaccine works differently for women with HIV. By doing this study, we hope to answer these questions.

Why am I being asked to participate?
You are being asked to take part in this study because you are a woman, aged 18-28 years of age, physically and mentally able and willing to participate in the study, and able and willing to provide written, informed consent, or else consent with a thumbprint.

What will happen if I participate in the study?
Today, in a private location at the study clinic you will complete a short questionnaire about your life, your reproductive health, and your sexual history. All your answers are strictly confidential. You do not have to respond to any question that makes you uncomfortable. You will provide a urine sample for a pregnancy test to confirm you are not pregnant. If you do not have HIV, you will take a rapid HIV test with a finger prick. A study nurse will provide counseling for you to understand what the results mean for you. A study nurse will collect a sample of your blood into a collection tube for testing on how your body responds to the HPV vaccine and possible infection. In a private exam room, the study nurse will help you swish a saline solution in your mouth and then spit it into a container. To collect cells from your vagina, the study nurse will insert a soft brush into your vagina, turn it three times and remove it. To collect cells from your anal canal the study nurse will insert a small swab into your anal canal, turn it for 20 seconds and remove it. Both collections are quick procedures with minimal discomfort. If all three samples do not have HPV, you are done with the study visits. If any of these three samples has HPV, we will ask you to return in 6-12 months for another visit so you can provide samples again and see if you still have HPV or if it has gone away on its own. If you still have HPV, we may ask you to see a special doctor to make sure you do not have precancer. At your visit with the special doctor, they will take one or more digital pictures of your cervix with a digital camera. The picture will not show the outside of your body, just the inside where the cervix is. Please note that the pictures will not identify you, and that picture of a cervix will not show your face or genitals. People cannot tell the specific woman from whom the cervix picture is taken. All pictures will be shown to you at the time if you want to see them.

How many people will take part in the research study?
You will be one of approximately 3,028 women participating in this study. We will be enrolling **1,514** women living with HIV and **1,514** women who are not infected with HIV.

Will there be audio and/or video recording?
No, there will be no audio or video recording in this study. You will hear an audio recording for the questionnaire that will be completed in private.

Information Banking (Future Use and Storage)

- No testing of human genes will be conducted as part of the current study.
- ADDITIONAL TESTS ON YOUR SAMPLE: No other tests other than those explained under this study are currently planned.

However, if you agree, the specimens you provide for this research may be stored for future research testing. The specimen cannot be linked to you. In the future, researchers can apply for permission to use the specimen for new studies to prevent, diagnose or treat disease. Future testing may require samples to be analyzed in Rwanda, the United States, or elsewhere for testing. Your specimens/data may be kept for a long time, perhaps longer than 50 years.

If you agree to the future use, some of your de-identified specimens, digital pictures of your cervix and health information (not linked to you) may be placed into one or more scientific databases. Your specimens may also be submitted to a tissue/cell/DNA bank. You have the right to withdraw consent to use of the tissue for future use at any time by contacting the study doctors listed above. If that is your request, your unused specimens will be destroyed at the end of this study. Some researchers may develop tests, treatments or products that are worth money. You will not receive payment of any kind for your specimens/data or for any tests, treatments, products, or other things of value that may result from the research.

You can choose not to participate in the tissue/cell/DNA bank and still be part of the main study and this will not affect your treatment at this facility.

PLEASE INDICATE YOUR CHOICE BY INITIALING ONE (1) OF THE FOLLOWING OPTIONS FOR EACH QUESTION

1. Specimens

_____ I consent to have my specimens stored and used for future research studies.
_____ I do NOT consent to have my specimens stored and used for future research studies. The specimens will be destroyed at the end of the study.

2. Future Contact About This Study

____I consent to be contacted with general information about this study's research findings.
____I do NOT consent to be contacted with general information about this study's research findings.

3. Future Research

___I consent to being contacted about future research opportunities.
___I do NOT consent to being contacted about future research opportunities.

Will I be compensated for being in this research study?

You will receive a total of 10,000 RWF in cash to compensate for your time and transport costs during the study visit and other follow-up visits if necessary. If you choose to withdraw from the study before all components of your participation are completed, you will be compensated only for the components you completed.

Will it cost me anything to participate in this study?

There will be no cost to you to participate in the study.

Confidentiality

The researchers and study staff follow US federal, US state, and Rwandan laws to protect your privacy. This part of the consent form tells you what information about you may be used and shared in the research described in this form. You do not have to sign this form but, if you do not, you may not participate in the research.

The health information that we may use or disclose for the research described in this form includes information from your entire medical record, such as your name, phone number, email, medical diagnoses, dates, test results, social security number, medical record numbers, etc. In addition, the researchers wish to review information from your medical records pertaining to your HIV status and previous clinical care. If you are living with HIV or test positive for HIV today, the study staff may review information about your current and previous HIV care and laboratory tests from the health clinic. By law, you must specifically authorize access to these records:

☐ Yes, I authorize the use and disclosure of my information pertaining to HIV testing and HIV status.

Initial: _____ Date: _____

Your information and research records will be kept confidential. Your study information will be kept if they are useful for the research described in this form. The only people who can see your research records are:

- Researchers and other individuals who work with the researchers
- Organizations and institutions involved in this research, including those that fund the research, if applicable
- Groups that review research such as central reviewers, Institutional Review Boards, the Office for Human Research Protections, the US Food and Drug Administration, data coordinating centers, and domestic and foreign agencies that regulate research.

The purposes of these uses and disclosures are to (1) conduct the study and (2) make sure the study is being done correctly. The information covered under this form may no longer be protected by federal privacy laws (such as HIPAA) once disclosed, and those persons who receive your health information may share your information with others without your additional permission. All these groups are required to keep your information confidential.

Certificate of Confidentiality

As a way to protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health, which is funding this study. If information from this study were requested or subpoenaed by government agencies or the courts, we would use the Certificate to attempt to legally refuse to provide that information. These requests are rare – in only a few cases did researchers have to use the Certificate, and it was honored most of the time, but not every time. There are several kinds of situations to which the Certificate does not apply. For example, we are still required to report child abuse and some diseases, and we must make data available to the government for review or evaluation of our research. The Certificate does not prevent you or a member of your family from voluntarily sharing information. Similarly, if an

insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

Are there any risks to me?
We do not think there are any physical risks related to participating in this research study. We will ask you questions about your sexual history and reproductive health that might make you feel uncomfortable. You have a right **not** to respond to any question that makes you uncomfortable.

A risk of taking part in this study is the possibility of a loss of confidentiality or privacy. Loss of privacy means having your personal information shared with someone who is not on the study team and was not supposed to see or know about your information. The study team plans to protect your privacy – see the Confidentiality section above for details.

However, there is occasionally the possibility of discomfort, bleeding, or bruising from the blood draw. These risks will be minimized by having a trained and experienced health worker draw your blood.

Are there possible benefits to me?
Unless you are known to have HIV, you will have a rapid test and learn whether you have HIV. It is very unlikely that you have cervical or anal precancer. By participating in this study, any precancer diagnosed would be treated. More likely, you will not experience any direct benefit personally from participating in this study. We hope you will participate because the study will generate important information contributing to the knowledge of HPV vaccine effectiveness and immunity in Rwanda women living with or without HIV. Your participation will help to inform the development of programs and interventions for communities of people like yourself.

Will I get to know the results from this study?
If you have an HIV test, you will learn those results immediately. The result of the HPV infection test performed on you will not be communicated directly to you. However, if any of the three samples become positive, you may be contacted for follow-up visits approximately six-twelve months later. And if, at follow-up visits, the HPV results remain positive, the study team may perform further tests to rule out precancer or refer you to the nearest specialized health service if there is any advanced disease. Some other results, which are obtained for research purposes only, will not be directly returned to you. However, the overall results of the research study will be made available to the public.

What choices do I have other than participating in this study?
You can refuse to participate in the study. If you decide not to participate, the medical care providers at this facility will still give you all standard care and treatment that is recommended for you.

Are there any consequences to me if I decide to stop participating in this study?
No. If you decide to take part, you are free to stop participating at any time without giving a reason. This will not affect your care and you will continue to be treated at this facility. However, some of the information may have already been entered into the study and that will not be removed. The researchers and the sponsor may continue to use and share the information they have already collected.

To withdraw your consent and authorization, you must contact the Principal or Co-Investigator in writing at the address on page 1 of this form. However, you may first call or speak to the Principal or co-Investigator and he/she will stop collecting new information about you. If you take back your consent and authorization, you will not be allowed to continue to participate in this research study.

Who can answer my questions about the study?
You can talk to the research team member that explains the study to you about any questions, concerns, or complaints. You may also telephone **Dr. Gad Murenzi / Dr. Fabienne Shumbusho** on **0788589085/0788559065.** If you wish to ask questions about the study or your rights as a research participant to someone other than the researchers or if you wish to voice any problems or concerns you may have about the study, please contact: RNEC chairperson **Dr. Mazarati Jean Baptiste** at (250) 78830987 or **Dr. Tumusiime David** at (250) 788749398 or by mail: info@rnecrwanda.org

Consent

You have been given a copy of this consent form.

Declaration

CONSENT TO PARTICIPATE

I have read the consent form and I understand that it is up to me whether I participate. I know enough about the purpose, methods, risks, and benefits of the research study to decide that I want to take part in it. I understand that I am not waiving any of my legal rights by signing this informed consent document. I will be given a signed copy of this consent form.

_____	_____	_____
Printed name of participant	Signature or thumbprint of participant	Date
_____	_____	_____
Printed name of the person conducting the consent process	Signature	Date

If the person is unable to read and/or write, an impartial witness should be present during the informed consent discussion. After the informed consent form is read and explained to the person, and after they have orally consented to their participation in the study and have either

signed the consent form or provided their fingerprint, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the person and that informed consent was freely given by the person.

Printed name of witness if participant utilized a thumbprint to consent to participation in this study	Signature of witness	Date
Printed name of the person conducting the consent process	Signature	Date

APPENDIX III. ENROLLMENT QUESTIONNAIRE

Thank you for agreeing to participate in our study. This interview should take no more than 20 minutes. Some of these questions are personal about your life, your reproductive health, and your sexual history. All your answers are strictly confidential, and it is very important for us that you try to answer all the questions as openly as possible, even if they are a little uncomfortable or embarrassing. This information will be important in understanding how the HPV vaccine protects women against HPV infection. If you don't understand any of the questions, please inform the study nurse who will try to help explain them. You should also be aware that you have a right not to respond to any question with which you are uncomfortable.

A. Socio-demographics

1. What is your date of birth? DD/ MM /YYYY (enter all 0 if not remembered)

_ _ / _ _ / _ _ _ _

2. What is your age? _ _ (Year)

3. What is your marital status?

- ❑ Married/Cohabiting
- ❑ Divorced
- ❑ Widowed
- ❑ Separated
- ❑ Single
- ❑ Choose not to answer

4. Do you live in Kigali?

- ❑ Yes
- ❑ No (Skip to Question #6)
- ❑ Choose not to answer (Skip to Question #6)

5. In what area in Kigali City Province do you live?

- ❑ Nyarugenge
- ❑ Kicukiro
- ❑ Gasabo urban (e.g., Kacyiru, Kimironko, Kinyinya, etc.) Gasabo rural (e.g., Nduba, Jabana, Gikomero, etc.)
- ❑ Do not know

❑ Choose not to answer

6. Who do you live with? (Check all that apply)

❑ Live alone
❑ Partner/spouse
❑ Daughter(s)/son(s)
❑ Parents and/or other family
❑ Other: _____

7. What is your primary occupation?

❑ Student
❑ Employed by government, another institution, or company
❑ Self-employed (Small and medium enterprises)
❑ Self-employed (High income earnings)
❑ Unemployed/Do not work
❑ Other (specify): _____
❑ Choose not to answer (Skip to Question #8)

The following questions are sensitive and personal in nature. Your answers will be kept confidential. You may choose not to answer certain questions. Answering any question is voluntary

B. Parity

8. What age did you have your first full term birth ? _ _ (Year) (Enter 00 if refuse to answer, enter 99 if never pregnant or not yet had a pregnancy that resulted in a full term birth – i.e. all pre-term deliveries) (if 00 or 99, skip to Question #11)

9. How many full term births have you had in your lifetime? _____

10. Have you given birth in the last year?

❑ Yes
❑ No
❑ Choose not to answer

C. Contraceptive Use

11. Have you ever used Jadell (contraceptive)?

❑ Never
❑ No (Skip to Question #13)
❑ Choose not to answer (Skip to Question #13)

12. Do you currently use Jadell (contraceptive)?

❑ Yes
❑ No
❑ Choose not to answer

13. Have you ever used oral contraceptives?

❑ Yes
❑ No (Skip to Question #15)
❑ Choose not to answer (Skip to Question #15)

14. Do you currently use oral contraceptives?

❑ Yes
❑ No
❑ Choose not to answer

15. Have you ever used Depo Provera (contraceptive)?

❑ Yes
❑ No (Skip to Question #17)
❑ Choose not to answer (Skip to Question #17)

16. Do you currently use Depo Provera (contraceptive)?

❑ Yes
❑ No
❑ Choose not to answer

17. Do use condoms as a contraceptive?

❑ Yes
❑ No (skip to Question #19)
❑ Choose not to answer (skip to Question #19)

18. Have you used condoms as a contraceptive in the last 6 months?

- ❑ Yes
- ❑ No
- ❑ Choose not to answer

19. Have you ever used another contraceptive method?

- ❑ Yes. Please specify: _____
- ❑ No (skip to Question #21)
- ❑ Choose not to answer (skip to Question #21)

20. Do you currently use another contraceptive method?

- ❑ Yes. Please specify: _____
- ❑ No
- ❑ Choose not to answer

D. Sexual Behaviors

This last section of the questionnaire contains questions about sexual practices you may have engaged in over the years. This will help us understand your likelihood of having been exposed to human papillomavirus (HPV).

21. How many men have you had vaginal sex with, in your lifetime until now? Vaginal sex is a man inserting his penis into your vagina.

- ❑ No Men (Skip to question #25)
- ❑ 1 Man
- ❑ 2-3 Men
- ❑ 4-5 Men
- ❑ 6 or more Men
- ❑ Choose not to answer

22. How old were you when you first had vaginal sex? _ _ (Year) (Enter 00 if refuse to answer)

23. How many men have you had vaginal sex with in the last 6 months?

- ❑ No Men (Skip to Question #25)
- ❑ 1 Man
- ❑ 2 or more Men

❑ Choose not to answer

24. In the past 6 months, how often has he used a condom during vaginal intercourse?

❑ Never
❑ Occasionally
❑ Half the time
❑ Almost always
❑ Always

25. Have you ever had anal intercourse? Anal intercourse is a man inserting his penis into your anus/rectum.

❑ Yes
❑ No (Skip to Question #29)
❑ Choose not to answer (Skip to Question #29)

26. How many men have you had anal sex with, in your lifetime until now?

❑ 1 Man
❑ 2-3 Men
❑ 4-5 Men
❑ 6 or more Men
❑ Choose not to answer

27. How many men have you had anal sex with, in the past 6 months?

❑ No Men (Skip to Question #29)
❑ 1 Man
❑ 2 or more Men
❑ Choose not to answer

28. In the past 6 months, how often has heused a condom during anal sex?

❑ Never
❑ Occasionally
❑ Half the time
❑ Almost always
❑ Always

29. Have you ever performed oral sex on a man? (Oral sex would be a blow job or you sucking someone's penis)

- ❑ Yes
- ❑ No (Skip to Question #32)
- ❑ Choose not to answer (Skip to Question #32)

30. In your lifetime until now, how many men have you performed oral sex on until now?

- ❑ 1 Man
- ❑ 2-3 Men
- ❑ 4-5 Men
- ❑ 6 or more Men
- ❑ Choose not to answer

31. During the past 6 months, how many men have you performed oral sex on?

- ❑ No Men
- ❑ 1 Man
- ❑ 2 or more Men
- ❑ Choose not to answer

E. HPV Vaccination History

32. Have you had the HPV vaccine (most women have the vaccine when they are around 11-13 years old)?

- ❑ Never vaccinated (End of Questionnaire)
- ❑ Vaccinated
- ❑ I don't remember (End of Questionnaire)

33. How many times have you had the HPV vaccine?

- ❑ One time
- ❑ Two times
- ❑ Three times
- ❑ I don't remember

END OF QUESTIONNAIRE

APPENDIX IV. FOLLOW UP QUESTIONNAIRE

Thank you for agreeing to participate in our study. This interview should take no more than 10 minutes. These questions are personal about your sexual history. All your answers are strictly confidential, and it is very important for us that you try to answer all the questions as openly as possible, even if they are a little uncomfortable or embarrassing. This information will be important in understanding how the HPV vaccine protects women against HPV infection. If you don't understand any of the questions, please inform the study nurse who will try to help explain them. You should also be aware that you have a right not to respond to any question with which you are uncomfortable.

1. What is your date of birth? DD/ MM /YYYY (enter all 0 if not remembered)

_ _ / _ _ / _ _ _ _

2. What is your age? _ _ (Year)

The following questions are sensitive and personal in nature about sexual practices you may have engaged in since your last study visit. This will help us understand your likelihood of having been newly exposed to human papillomavirus (HPV). Your answers will be kept confidential. You may choose not to answer certain questions. Answering any question is voluntary.

3. Since your last visit, how many men have you had vaginal sex with?

- ❑ No Men
- ❑ 1 Man
- ❑ 2 or more Men
- ❑ Choose not to answer

4. Since your last visit, how many men have you had anal sex with?

- ❑ No Men
- ❑ 1 Man
- ❑ 2 or more Men
- ❑ Choose not to answer

5. Since your last visit, how many men have you performed oral sex on?

- ❑ No Men
- ❑ 1 Man
- ❑ 2 or more Men

❑ Choose not to answer

END OF QUESTIONNAIRE

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