A multi-site, open-label non-inferiority trial to assess immunogenicity of two doses of the nonavalent human papillomavirus (HPV) vaccine among children, adolescents and young adults (9-26 years) living with HIV vs three doses of nonavalent HPV vaccine among HIV uninfected young women (15-26 years) in Eswatini

Protocol Version 4.0

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Abbreviations and Acronyms

AE	adverse event
AIDS	acquired immunodeficiency syndrome
ANOVA	analysis of variance
ART	antiretroviral treatment
CD4	cluster of differentiation 4
CDC	U.S. Centers for Disease Control and Prevention
cLIA	clinical laboratory improvement amendments
GAVI	Global Alliance for Vaccines and Immunization
GMT	geometric mean titers
HIV	human immunodeficiency virus
HPV	human papilloma virus
ICH/GCP	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use/Good Clinical Practice
IgG	immunoglobulin
JAIDS	Journal of Acquired Immune Deficiency Syndrome
JAMA	Journal of the American Medical Association
MISP	Merck Investigator Studies Program
mL	milliliter
MoH	Ministry of Health
MSD	Merck Sharp & Dohme Corporation
NEJM	The New England Journal of Medicine
OGAC	Office of the U.S. Global AIDS Coordinator
PEPFAR	U.S. President's Emergency Plan for AIDS Relief
PSM	Process Safety Management
RNA	ribonucleic acid
SAE	serious adverse event
WHO	World Health Organization

Protocol signature page

HPV Vaccine Study: Protocol version 4.0, dated 15 September 2022
Clinical Trials Registration number: NCT04982614

A multi-site, open-label non-inferiority trial to assess immunogenicity of two doses of the nonavalent human papillomavirus (HPV) vaccine among children, adolescents and young adults (9-26 years) living with HIV vs three doses of nonavalent HPV vaccine among HIV uninfected young women (15-26 years) in Eswatini

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US.) Health and Human Service regulations (45 CFR 46); applicable US. Food and Drug Administration regulations; standards of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice (E6) (R2); Institutional Review Board/Ethics Committee determinations; all applicable in-country and local laws and regulations; and other applicable requirements.

I have read and understand the information in this protocol and will ensure that all collaborators, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

_____

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Protocol Synopsis

Protocol Title	A multi-site, open-label non-inferiority study to assess immunogenicity of two doses of the nonavalent human papillomavirus (HPV) vaccine among children, adolescents and young adults living with HIV versus three doses of HPV vaccine among HIV uninfected young women in Eswatini.
Background	Cervical cancer remains a significant threat to public health worldwide and remains the leading cause of cancer-associated mortality in women in Sub Saharan Africa. Cervical cancer-associated morbidity and mortality is preventable through HPV vaccination, and screening and treatment of pre-cancerous lesions. As the primary cause of cervical cancer, HPV is a vaccine-preventable infection, with quadrivalent (4vHPV) and nonavalent (9vHPV) vaccines demonstrating 95% efficacy in immunocompetent women. Efficacy data of HPV vaccine is based on referenced data from a 3-dose regimen among young women 15-26 years. Non-inferior, often higher, antibody levels have been demonstrated in 9-14 year-old boys and girls after two-dose 9vHPV vaccine regimens compared to levels in 15-26 yearold women after three-dose regimens. Immunogenicity and safety of the two-dose regimen among people living with HIV (PLHIV) has not been reported. To date, there is no nationwide HPV vaccination program in Eswatini. Eswatini is not eligible for Global Alliance for Vaccines and Immunization (GAVI) procurement of HPV vaccines, and yet the triad of HIV, STI and cervical cancer generates a severe disease burden warranting immediate introduction of this intervention. Given the global limitations in vaccine supply and the high cost for procurement, country programs, including in Eswatini, must optimize vaccine resources in order to maximize HPV vaccine coverage. This protocol seeks to assess immunogenicity of a two-dose 9vHPV vaccine regimen among girls and boys (9-14 years) and young women (15-26 years) living with HIV on antiretroviral therapy versus a three-dose 9vHPV vaccine regimen among HIV uninfected young women (15-26 years) in Eswatini. This HPV vaccination project will include two collaborative protocols led by the Eswatini Ministry of Health (MOH) and implemented by ICAP at Columbia University (Protocol 1) and Baylor College of Medicine Children's Foundation Eswatini (Protocol 2). This document describes Protocol 1 activities only.
Primary Objectives	1. To test non-inferiority in immunogenicity between two doses of the 9vHPV vaccine in females (ages 9-26 years) and males (ages 9-14) living with HIV infection vs. three doses of the 9vHPV vaccine in HIV uninfected young women (15-26 years) in Eswatini.
Secondary Objectives	1. To examine the safety profiles of the two-dose 9vHPV regimen in females (ages 9-26 years) and males (9-14 years) living with HIV infection and three-dose 9vHPV regimen in HIV uninfected young women (15-26 years) in Eswatini.

	2. To measure completion of vaccination series of the 9vHPV vaccine among females and males (9-14 years) living with HIV infection, and among HIV-infected and uninfected young women (15-26 years). 3. Evaluate immunogenicity after 1 dose of 9vHPV vaccine among male and female adolescents 9-14 years and female youth 15-26 years living with HIV
Methods and Procedures	This is a multi-site, open-label non-inferiority study of the 9vHPV vaccine among a population of children, adolescents and young women living with HIV in Eswatini. The study will compare immunogenicity of two doses of the 9vHPV vaccine among girls and boys (9-14 years) and young women (15-26 years) living with HIV on antiretroviral therapy vs. three doses of 9vHPV vaccine among HIV uninfected young women (15-26 years). The study will also measure adverse events as well as completion of vaccine series among participants. The study will include 9vHPV vaccinations at 0 and 6 months (two-dose regimen) and at 0, 2, and 6 months (three-dose regimen). For all participants receiving the two-dose regimen, a delayed standard of care vaccine dose will be given at 12 months. Immunogenicity endpoint will be assessed 4-weeks post 6-month vaccine dose. As a secondary objective, we shall also review immunogenicity in samples collected before the second vaccine dose among PLHIV. Adverse events and vaccine completion will be assessed after 0 and 6 months (two-dose regimen) and at 0, 2, and 6 months (three-dose regimen).
Study Population	Approximately 1,400 participants followed for approximately 1 year. This will include: • PLHIV who will receive a two-dose regimen of the 9vHPV vaccine: males 9-14 years (N=350), females 9-14 years (N=350), and females 15-26 years (N=350) • A reference group of HIV uninfected females 15-26 years, who will receive a three-dose regimen of the 9vHPV vaccine (N=350)
Primary Endpoints	Aligned with study objectives, endpoints will include: • Anti-HPV geometric mean antibody titers (GMTs) assessed by cLIA at month 7 (4-weeks post 6-month vaccine dose) • Number of participants who seroconvert (no antibodies pre-vaccination, but detectable antibodies at 4 weeks post month 6 vaccine dose)
Secondary Endpoints	• Number of participants with serious adverse events • Number of participants completing 0, 2, and 6 month vaccinations, depending on assigned regimen • Anti-HPV geometric mean antibody titers (GMTs) assessed by cLIA at about month 6 after the first vaccination dose among PLHIV (to be conducted using samples collected prior to month 6 dose administration)
Timeline	PLHIV receiving the 2-dose regimen: will be followed for approximately 1 year after the first dose of 9vHPV vaccination. A reference group of HIV uninfected females 15-26 years receiving the 3-dose regimen: will be followed for 7 months after the first dose of 9vHPV vaccination.

Collaborating Institutions

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b. Collaborators

Collaborators: Investigators will collaborate with the following individuals and institutions during the course of the study, this may include consultation on issues around data, laboratory and vaccine management etc.		
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Funding

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1. Background

1.1 Disease Burden of Cervical Cancer and HPV infection

Cervical cancer remains a significant threat to public health worldwide. Annually, over half a million women are diagnosed with cervical cancer, with over 300,000 deaths estimated per year [1-3]. Low- and middle-income countries carry the highest cervical cancer disease burden [4], and account for 85% of deaths [5]. In sub-Saharan Africa, cervical cancer is the leading cause of cancer-associated mortality in women: approximately 100,000 women are diagnosed annually and 62% of those diagnosed will die from the disease [1, 3]. Moreover, cervical cancer in women living with HIV infection is especially alarming as they are up to 6 times more likely to develop cervical pre-cancerous and cancerous lesions, which lesions are often with more aggressive forms associated with high mortality rates [4, 6-9]. In many sub-Saharan African countries, HIV has worsened the cervical cancer disease burden [6, 7, 10, 11].

In Eswatini, cervical cancer is the leading malignancy in women of reproductive age and the leading cause of death in women 15-44 years of age [1]. In a 6-year period from 2012 to 2018, cervical cancer mortality increased from 53% to 67% 2018 [1]. The age-standardized cervical cancer incidence exceeds 60 per 100,000 in Eswatini [1, 12], and it is one of the highest in the world [1]. The added burden of HIV among young women in Eswatini, where HIV prevalence exceeds 30% in the reproductive age group [13-15], poses a serious public health concern.

Nearly 100% of cervical cancers are caused by HPV infection [16]. The cumulative lifetime risk for HPV infection in sexually active women exceeds 50%, and HPV infection is also very prevalent in men [2, 17]. Estimates of HPV infection are limited in many African countries. In Eswatini, Ginindza et al. estimated that high risk HPV prevalence was 46.2% among a cohort of 15-49 year old women from 5 health facilities [18]. Furthermore, this analysis found that overall high-risk HPV/HIV co-infection was 24.4% and significantly higher among younger women [18].

1.2 HPV Vaccines

Cervical cancer-associated morbidity and mortality is preventable through HPV vaccination, and screening and treatment of pre-cancerous lesions. As the primary cause of cervical cancer, HPV is a vaccine-preventable infection. There are currently three HPV vaccines licensed by the Food and Drug Administration (FDA) and European Medicines Agency (EMA): the bivalent, quadrivalent (4vHPV) and nonavalent (9vHPV) vaccines, all with high efficacy [19, 20]. Among immunocompetent women, clinical efficacy of 95% has been observed with the 4vHPV and 9vHPV vaccines, with the 9vHPV vaccine estimated to prevent 20% more cervical cancers than the 4vHPV vaccine [19]. Although uptake of HPV vaccination is still sub-optimal globally [21], countries that have scaled-up HPV vaccination have observed marked declines in cervical cancer incidence [19, 22, 23], and modelling data support this observation as well [12, 24].

Current available HPV vaccines have also been found to be well tolerated, with a excellent safety profile [25-27]. Adverse reactions in HPV vaccine clinical trials were observed among about 1% of recipients with few individuals discontinuing study participation due to adverse experiences after receiving the vaccine. Serious adverse events were reported in about 2% of the population.

While the safety and efficacy of the HPV vaccine well documented among healthy individuals, additional research in people living with HIV (PLHIV) is needed. In a small phase III study including 100 PLHIV 18-45 years, three doses of the 9vHPV vaccine had acceptable safety and immunogenicity outcomes [28]. Among children living with HIV, similar observation were found in another study of 178 adolescent boys and girls in Kenya, where 3 doses of the 4vHPV vaccine was efficacious and well tolerated, with better outcomes among PLHIV with higher CD4-counts (200+ cells/μL) and among those virally suppressed [29].

Efficacy data of HPV vaccine is based in reference data from a 3-dose regimen among young women 15-26 years [30, 31]. However, non-inferior, often higher, antibody levels have been demonstrated in 9-14 year old boys and girls after two-dose 9vHPV vaccine regimens compared

to levels in 15-26 year old women after three-dose regimens [32], with sustained antibody response following vaccination demonstrated up to 8 years post vaccination [25, 32, 33].

Evaluation of the efficacy of the two-dose regimen among PLHIV has not been reported, yet such data could impact the policy and pace of vaccinations among less resourced countries. Should the two-dose regimen be non-inferior to the three-dose regimen, countries with high HIV prevalence like Eswatini could potentially vaccinate 33% more individuals with the same amount of HPV vaccine. In support of efforts to introduce HPV vaccination in Eswatini and optimize vaccine coverage, we propose to evaluate immunogenicity, adverse events, and uptake of a two-dose regimen of the 9vHPV vaccine among boys, girls and young women living with HIV and on antiretroviral therapy compared to three doses of the 9vHPV vaccine in HIV uninfected young women (15-26 years) in Eswatini.

In April 2022 a WHO Strategic Advisory Group of Experts (SAGE) held an immunization meeting and advised that countries may now choose between a one- or two-dose schedule for 9–14-year-old girls for programmatic purposes[1]. This proposed schedule is to promote expanded access to vaccination in lower-resource settings, with comparable individual protection among immune competent individuals. The SAGE noted the dearth of data among immunocompromised individuals such as PLHIV, and recommended that such individuals age 9 years and older should be prioritized to receive at least two doses. For such individuals, three doses are considered optimal if programmatically feasible. As immunogenicity data among immunocompromised individuals such as PLHIV are still needed, we propose to evaluate immunogenicity after 1 dose of 9vHPV vaccine among male and female adolescents 9-14 years and female youth 15-26 years living with HIV, prior to administration of the second dose at 6 month. This is a particularly important area to assess in Eswatini, due to the enormous HIV disease burden. HIV prevalence among young women 20-24 years is 21%, and more than half of females aged 30-44 years are

[1] World Health Organization, Highlights from the Meeting of the Strategic Advisory Group of Experts (SAGE) on Immunization 4-7 April 2022. https://www.who.int/news/item/11-04-2022-one-dose-human-papillomavirus-(hpv)-vaccine-offers-solid-protection-against-cervical-cancer

living with HIV[2]. These additional data would inform the 1-dose roll out strategy in countries like Eswatini, which have a high HIV disease burden among adolescents and youth.

1.3 Public Health Significance

To date, there is no nationwide HPV vaccination program in Eswatini. Eswatini is not eligible for Global Alliance for Vaccines and Immunization (GAVI) procurement of HPV vaccines, and yet the triad of HIV, STI and cervical cancer disease burden warrants immediate introduction of this intervention. Given the global limitations in vaccine supply and the high cost for procurement, country programs must optimize vaccine resources to achieve optimal HPV vaccine coverage nationally. If this study demonstrates non-inferior immunogenicity for two- vs three-dose regimen, the findings provide evidence to support a two-dose regimen for boys and girls and young women living with HIV, potentially saving 33% of the vaccine supply in order to broaden vaccination campaigns. A combined strategy of HPV vaccination together with screening and treatment of pre-invasive lesions has the potential to eliminate cervical cancer by the end of this century [34].

2. Objectives

The study objectives are:

Primary Objective:

1. To test non-inferiority in immunogenicity between two doses of the 9vHPV vaccine in females (ages 9-26 years) and males (ages 9-14) living with HIV infection vs. three doses of the 9vHPV vaccine in HIV uninfected young women (15-26 years) in Eswatini.

Secondary Objectives:

1. To examine the safety profiles of the two-dose 9vHPV regimen in females (ages 9-26 years) and males (9-14 years) living with HIV infection and three-dose 9vHPV regimen in HIV uninfected young women (15-26 years) in Eswatini.
2. To measure completion of vaccination series of the 9vHPV vaccine among females and males (9-14 years) living with HIV infection, and among HIV-infected and uninfected young women (15-26 years).).

[2] Government of the Kingdom of Eswatini. Swaziland HIV Incidence Measurement Survey 2 (SHIMS2) 2016-2017. Final Report. April 2019.

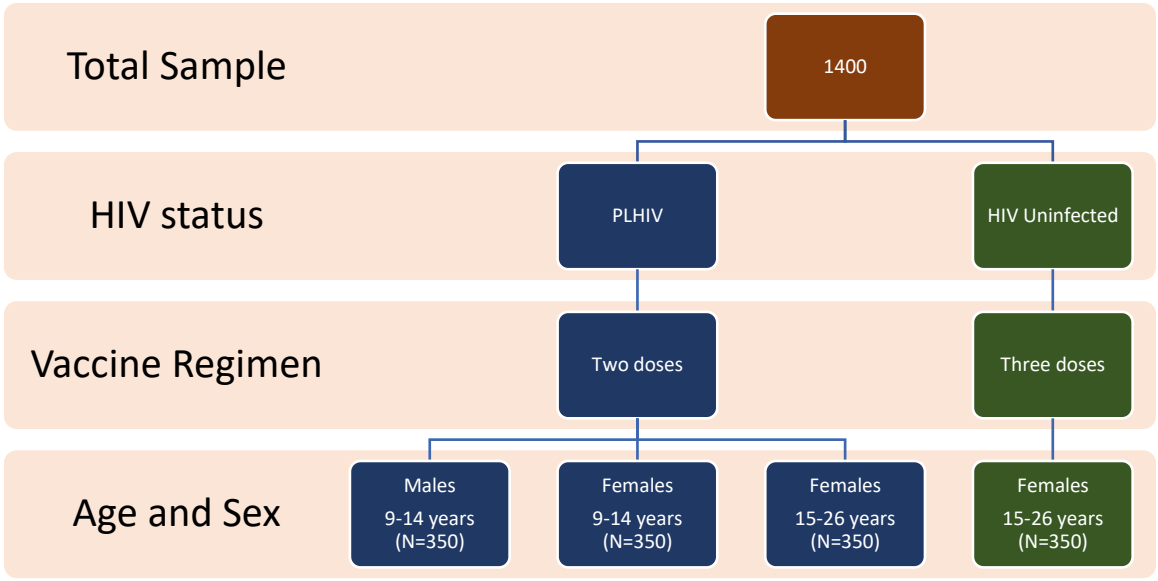
3. Evaluate immunogenicity after 1 dose of 9vHPV vaccine among male and female adolescents 9-14 years and female youth 15-26 years living with HIV

3. Study Methods

3.1 Study Design

This is a multi-site, open-label non-inferiority study of the 9vHPV vaccine among a population of children, adolescents and young women living with HIV in Eswatini. The study will compare immunogenicity of two doses of the 9vHPV vaccine in females (ages 9-26 years) and males (ages 9-14) living with HIV infection versus three doses of 9vHPV vaccine in HIV uninfected young women (ages 15-26) in Eswatini. The study will include 9vHPV vaccinations among PLHIV at 0 and 6 months (two-dose regimen), and at 0, 2, and 6 months (three-dose regimen) among HIV uninfected young women (See Figure 2). Immunogenicity will be assessed at approximately 6 months before the second vaccination dose is administered (among PLHIV only) and at 7 months for all participants (after two doses among PLHIV and 3 doses among HIV negative participants). Adverse events and vaccine series outcomes will be assessed after 0 and 6 months (two-dose regimen) and at 0, 2, and 6 months (three-dose regimen). PLHIV in the two-dose regimen arms will receive a third dose of 9vHPV vaccination at approximately 12 months to align with the current standard of care.

Figure 2: Study Design



3.2 Study Sites and Establishment of Study Cohorts

The study sites were purposively selected by the Eswatini Ministry of Health (MOH). The main eligibility criterion for site selection was ability to recruit the target population according to the number of girls, boys and young women living with HIV enrolled in HIV care. Using Ministry of Health routine monitoring and evaluation data, four potential sites have been identified for this study: Baylor College of Medicine Children's Foundation Eswatini (including Baylor satellite clinics at Raleigh Fitkin Memorial and Hlathikhulu) AHF Lamvelase clinics, Mbabane Government and Good Shepherd hospitals and public health units. Recruitment of HIV negative women will target adolescent and young women accessing sexual and reproductive health services. For efficiency, recruitment will start primarily at Baylor clinics (PLHIV, prioritizing children, adolescents, and young adults) and AHF Lamvelase clinics (the rest of PLHIV and HIV uninfected women). Recruitment will be expanded to the additional sites named above as needed to reach the target samples by age and sex, as shown in Figure 2.

3.3 Study Population and Sample Size

The study will include PLHIV: males 9-14 years (n=350), females 9-14 years (n=350), and females 15-26 years (n=350), and HIV uninfected females 15-26 years (n=350). The inclusion and exclusion criteria are shown in Table 1.

Table 1: Inclusion and Exclusion Criteria

PLHIV receiving two-dose regimen	
Inclusion Criteria	**Exclusion Criteria**
a) Females 9-26 years and males 9-14 years, b) Documented HIV infection, c) Aware of HIV+ status d) Currently receiving antiretroviral treatment (ART) for HIV infection for at least six months, e) Living in Eswatini, f) For individuals 18+ years: able to provide informed consent, g) For individuals 15-17 years: able to provide informed assent, h) For individuals 9-14 years of age: able to provide informed assent AND accompanied by an adult caregiver who is able to give informed consent, i) Intending to remain in the vicinity of the study sites for the study period	a) Diagnosis of other immunosuppressive diseases or receipt of immunosuppressive medications, b) Diagnosis of other acute or chronic illness, c) Receipt of other vaccinations within 2 – 3 weeks before or after study vaccination, d) Receipt of blood-derived products within 6 months before or during the study, e) Previous receipt of an HPV vaccine, f) Currently pregnant, g) Known allergies to a vaccine component
Reference group of HIV-uninfected women 15-26 years receiving the three-dose regimen	
Inclusion Criteria	**Exclusion Criteria**
a) Females 15-26 years, b) Documented negative HIV test at baseline c) Living in Eswatini, d) Able to provide informed assent (15-17 years) or informed consent (18-26 years) e) Intending to remain in the vicinity of the study sites for the study period	a) Diagnosis of other immunosuppressive diseases or receipt of immunosuppressive medications, b) Diagnosis of other acute or chronic illness, c) Receipt of other vaccinations within 2 – 3 weeks before or after study vaccination, d) Receipt of blood-derived products within 6 months before or during the study, e) Previous receipt of an HPV vaccine, f) Currently pregnant, g) Known allergies to a vaccine component

Determination of HIV and ART status: For PLHIV participants, HIV status and ART use will be obtained from medical records at participating health facilities. The date of HIV diagnosis will be

abstracted, together with the date of ART initiation. Established procedures used in this setting and detailed in site-level SOPs will be used to determine individuals on ART for 6 or more months from the date of eligibility screening. For the HIV negative reference group, HIV status will be determined through rapid testing following the national HIV testing algorithm on the day of eligibility screening.

Definition of sex and age categories: Participant sex will be recorded according to sex at birth. To define age categories, age will be determined on the day that eligibility screening is conducted, taking the age at last birthday. An age calculator will be used to standardize age categories. Where possible, the full date of birth should be obtained from appropriate records e.g. birth certificate or national identification card; and compared to the date of eligibility screening. Where date of birth is unknown, the latest possible date in the month will be considered for determination of date of birth. Where month of birth is unknown, the latest possible month (December) will be considered as the month of birth. A note should be made to indicate that date and/or month of birth was estimated, or if the date of birth is by participant/guardian report (without documentation). Participants with unknown, year of birth will be excluded from the study. The following age-categories will be used for the study:

- **9-14 years:** on date of eligibility screening, participants who are 9 years up to 14 years, 11 months, and 29 days at the last birthday.
- **15-26 years**: on date of eligibility screening, participants 15 years up to 26 years at the last birthday.

3.4 Study Endpoints

The study has multiple endpoints corresponding to specific study objectives. The primary endpoint is immunogenicity assessed at approximately 1 month post Month 6 vaccination. Table 2 below summarizes the objectives and associated endpoints.

Table 2: Study Objectives and Associated Endpoints

Primary Objective	Endpoint
To test non-inferiority in immunogenicity between two doses of the 9vHPV vaccine in females (ages 9-26 years) and males (ages 9-14) living with HIV infection vs. three doses of the 9vHPV vaccine in	• Anti-HPV geometric mean antibody titers (GMTs) assessed by cLIA at month 7 (4-weeks post 6-month vaccine dose)

HIV uninfected young women (15-26 years) in Eswatini.	• Number of participants who seroconvert (no antibodies pre-vaccination, but detectable antibodies at 4 weeks post month 6 vaccine dose)
Secondary Objectives	**Endpoints**
To examine the safety profiles of the two-dose 9vHPV regimen in females (ages 9-26 years) and males (9-14 years) living with HIV infection and three-dose 9vHPV regimen in HIV uninfected young women (15-26 years) in Eswatini.	• Number of participants with serious adverse events after each vaccination
To measure completion of vaccination series of the 9vHPV vaccine among females and males (9-14 years) living with HIV infection, and among HIV-infected and uninfected young women (15-26 years).	• Number of participants completing 0, 2, and 6 month vaccinations, depending on assigned regimen
To evaluate immunogenicity after 1 dose of 9vHPV vaccine among male and female adolescents 9-14 years and female youth 15-26 years living with HIV	Anti-HPV geometric mean antibody titers (GMTs) assessed by cLIA at about month 6 after the first vaccination dose among PLHIV (to be conducted using samples collected prior to month 6 dose administration)

3.5 Beginning and End of the Study

The overall study begins when the first participant signs the informed consent form. The overall study ends when the last participant completes the last study visit, discontinues from the study or is deemed lost to follow-up (i.e., the participant is unable to be contacted by the study team).

4. Study Procedures

4.1 Community and participant engagement

To ensure study-related information is communicated to local stakeholders and potential participants, targeted stakeholder engagement will be conducted. The community engagement will largely be health-facility based, and include the following activities:

a) Sensitization and education through investigator meetings, site level meetings and trainings for health workers and health talks targeting potential participants at study sites. These sessions will include information, education, and communication materials such as the leaflet provided in Appendix 1.

b) Stakeholders will be provided regular updates on protocol development and implementation through existing MOH platforms such as technical working group meetings, study advisory group meetings and core leadership group meetings
c) Participants retention will be promoted through study provision of study appointments, and reminder phone calls for upcoming and missed study visits.
d) Investigators contact details will included in the ICF and participants will be allowed to retain a copy of the ICF. Any participants who have questions and concerns about study participation will be invited to contact the individuals listed on the ICF's.

4.2 Recruitment

Healthcare workers and authorities based at participating study sites and in the recruitment areas be sensitized about the study. Recruitment sites will include participating health facilities, other health facilities, learning institutions, and community settings. With the facilitation and collaboration of institutional authorities, study team members will provide basic information about the study, and invite interested individuals to visit the study sites for additional information. Media platforms such as print media and broadcasting media (radio and television talk shows) will also be utilized to create study awareness using the material in Appendix 13. For individuals 15 years and older, clinic staff will present the study directly to the adolescent/young adult and individuals who express interest in learning more about the study. Potential participants will be referred to designated research staff who will provide additional information and for those who are interested, will screen clients using the eligibility checklist.

For children and adolescents 9-14 years of age, clinic staff will inform their adult caregivers of the study. If caregivers are interested, clinic staff will refer them and their children to designated research staff. The research staff will take the caregiver and the adolescent through the consent/assent process and provide details about the study procedures. The child/adolescent will only be eligible to participate if both their caregiver and the child/adolescent agree to participation.

This recruitment strategy will be supplemented by providing details about the study to all potential participants in the waiting areas during the morning health talk. The healthcare worker giving the health talk will request those interested in learning more information about the study to proceed

through a designated area where study staff will conduct eligibility screening and informed consent/assent.

4.3 Eligibility screening and informed consent

Study staff will use an eligibility checklist (Appendix 2a/2b) to review potential participant eligibility against the protocol inclusion and exclusion criteria described above. Potentially eligible participants will then be taken through the informed consent and assent process as follows:

- Boys and girls/adolescents 9-14 years living with HIV will be requested for assent in addition to caregiver permission to participate (Appendix 3a and 3b).
- Adolescents 15-17 years will be requested to independently assent for participation. A waiver of caregiver consent will be requested for this age group (Appendix 3c and 3d).
- Young women 18-26 years will be requested to provide consent to participate (Appendix 3c and 3d).

4.4 Baseline/Enrollment Visit (Month 0)

Locator information

Study staff will collect complete locator information from each participant, including name, address, email and social media details, telephone number, and at least one alternative contact address and phone number that the participant feels comfortable providing.

Baseline questionnaire

Following informed consent/assent, an interviewer-administered structured questionnaire will be administered to all participants. This questionnaire will include demographic variables and questions on medical history, behavior, and perceptions. The study questionnaires are included in Appendix 4a.

Laboratory Tests

A sample of blood (approximately 10mL) will be obtained for testing for antibody titers.

For PLHIV: An additional 10mL for HIV viral load and CD4+ lymphocyte testing (only among those PLHIV who have not had viral load and CD4 conducted in the past 30 days, approximately).

For HIV uninfected women: Trained study staff will conduct HIV rapid testing using blood collected by finger stick or venous blood draw the study site. HIV testing and counseling will be

provided according to national guidelines using rapid antibody tests available on site, according to the national HIV testing algorithm. Individuals with one negative rapid antibody test (e.g. Determine TM) are considered HIV uninfected and eligible to be enrolled in the study if all other eligibility criteria are met. Individuals with two positive rapid antibody tests (e.g. Determine and Unigold) will be counseled on the importance of HIV care and rapid initiation of treatment for HIV and of remaining in care for life-long management of HIV infection and will be actively linked to a local health facility where HIV services are readily available. Individuals with an indeterminate or inconclusive HIV test results will be excluded from the study.

For all female participants: approximately 10mL ml urine sample will be taken for pregnancy testing.

HPV Vaccination

The study product, 9vHPV will be administered according to the sponsor guidelines articulated in the packet insert [31]. Participants will receive 9-valent HPV L1 VLP vaccine as a 0.5-mL intramuscular injection with the deltoid muscle of the non-dominant arm the preferred site of vaccination.

For female participants who have an implanted contraceptive at the time of the study vaccination, the study team will avoid administering the study vaccination in the arm with the implanted contraceptive. For those female participants have received injectable contraceptives or implanted contraceptives in both arms in the past 9 months, the injection will be given in the thigh rather than in an arm. Injections will not be given within 2 cm of a tattoo, scar, or skin deformation.

All participants will be counseled on symptoms to be reported to the study staff and given contact information for study personnel. Participants will also be told that a member of the study team will call them via phone 7-14 days post vaccination to assess any adverse events. Participants will be encouraged to reach out to the study staff with concerns or questions.

To promote review of the above procedures and protocol adherence, the vaccination form (Appendix 5) will include a check list to review these criteria and document the vaccination process.

If the participant does not meet the requirements for vaccination, the study visit (including specimen collection and study vaccination) will be re-scheduled.

Documentation of vaccination and appointments

All vaccinated participants will be given a vaccination card as evidence of their vaccination, and a reminder for the next study appointment. If available, the MOH standard vaccination card will be used (Appendix 6). In case of MOH stock-out of vaccination cards, a similarly designed study vaccination card will be utilized (see illustrative example in Appendix 6). Vaccine administration will also be documented in participant's routine medical record.

Data abstraction from routine medical records

For PLHIV, results from previous HIV viral load and CD4+ lymphocyte testing as well as data on current ART regimen data will be collected in the form of medical record review from records located at the study facilities. Permission to review medical records is included in informed assent and consent documents for all participants living with HIV. The data abstraction form in Appendix 7 will be used to collect these data.

4.5 Follow-up Vaccination Visits (Month 2, 6)

Schedule for follow-up vaccination visits including estimated window periods are detailed in Table 3. At Month 2 and Month 6, the injection administration form will be used to assess suitability for follow-on injections and pregnancy testing done. HIV negative participants without any contraindication to vaccination will receive vaccination. For all PLHIV, prior to second dose vaccination at month 6, a sample of blood (10ml) will be obtained for testing antibody titers.

4.6 Final Visit (Month 7)

Month 7 Questionnaire

At Month 7 visit, a questionnaire (Appendix 4b) will be administered to all participants, pregnancy testing done and vaccination administered to all participants without contraindications. This questionnaire will include questions related to the participant's general health status, sexual health, and experiences with HPV vaccination.

Laboratory Tests

A sample of blood (10 mL) will be obtained for testing for antibody titers.

For PLHIV: An additional 10mL for HIV viral load and CD4+ lymphocyte testing (only among those PLHIV who have not had viral load and CD4 conducted in the past 30 days, approximately).

For HIV uninfected women: Trained study staff will conduct HIV rapid testing using blood collected by finger stick or venous blood draw at the study site. HIV testing and counseling will be provided according to national guidelines (See section 4.4 above).

Individuals testing HIV-positive will be counseled on the importance of HIV care and rapid initiation of treatment for HIV and of remaining in care for life-long management of HIV infection and will be actively linked to a local health facility where HIV services are readily available.

Confirmation of Locator Information (for participants receiving two-dose regimen ONLY)

Study staff will confirm previously collected locator information from each participant, including name, address, email and social media details, telephone number and at least one alternative contact address and phone number that participant feels comfortable providing. Study staff will remind participants that they will be contacted by clinic staff to come back for the standard of care vaccination at Month 12.

Data abstraction from routine medical records

For PLHIV, results from previous HIV viral load and CD4+ lymphocyte testing as well as data on current ART regimen data will be collected in the form of medical record review from records located at the study facilities. Permission to review medical records is included in informed consent documents for all participants living with HIV.

Table 3: Proposed Visit Schedule and Visit Windows

Regimen	9vHPV vaccinations*	Vaccination Window	AE Window for Primary Safety End-Points
Three-dose (0, 2, 6)	Dose 1	Day 0	1-14 days post-dose
	Dose 2	M2 (until next scheduled visit)	1-30 days post-dose
	Dose 3	M6 ± 4 weeks	1-30 days post-dose
Two-dose (0, 6)**	Dose 1	Day 0	1-14 days post-dose
	Dose 2	M6 ± 4 weeks	1-30 days post-dose

* Should a participant miss their scheduled dose 2 or dose 3, and present after the allowable vaccination window, the missed dose can be administered at the next immediate contact, there is no need to re-start the vaccination schedule

**For all participants receiving the two-dose regimen, a delayed standard of care vaccine dose will be given at 12 months (± 4 wks)

4.7 Adverse Events (AEs) Assessments

Participants will generally receive their first vaccine dose at the enrollment visit (Month 0), and follow-up vaccinations will be given at Month 2 (three-dose regimen) and Month 6 (both regimens). AEs and SAEs will be **assessed via phone** in the 7-14 days after each vaccination. At the time of phone call, study staff will first verify responder details, and ask about any AEs experienced by the participant using the Adverse Events Form (Appendix 8). AEs will be assessed for intensity and if appropriate, seriousness, and likely relationship to 9vHPV vaccination using an adverse event assessment job aid. Outside of these planned phone calls, participants can self-report AEs/SAEs at any point during the study by calling designated study team members, or during clinic visits. These reports will also be captured on the Adverse Events Form.

4.8 Standard of Care Vaccine Dose for PLHIV at Month 12

For all participants receiving the two-dose regimen, a third dose will be given 12 months after the first dose to conform with the standard of care currently for those living with HIV and those 15-26 years old. Study staff will track timing of this 3rd dose and contact participants via phone to schedule Month 12 visit, in collaboration with clinic staff.

4.9 Pregnant and Breast-feeding Adolescent Girls and Young Women

All female subjects will have pregnancy testing done prior to vaccination. Pregnant women will not be eligible to participate in the study. For enrolled participants who become pregnant after receiving one or two study vaccinations, study visits and vaccinations will be paused until resolution of the pregnancy e.g., delivery or other termination. Vaccinations can be administered 4 weeks following the resolution of pregnancy. Breastfeeding is not a contraindication to receiving study vaccination.

4.10 Study Retention

Study team members will make efforts to promote study visit adherence and retention in the study. This will be through enrolling participants intending to remain in the vicinity of the study sites for the study period and providing reminder telephone calls/SMS messages prior to scheduled appointment and follow-up calls/SMS messages if participants miss visits. Additionally, for applicable participants, efforts will be made to align follow-up visits with routine HIV/ART clinic visits, in order to minimize transport costs for the participants.

4.11 Study Termination Criteria

It is anticipated that this study will be carried out to its conclusion (see section 3.5 on study duration). Study investigators will utilize protocol-defined indicators to monitor quality control and quality assurance (QC/QA). Such indicators include accrual rates, ensuring participants are appropriately consented, appropriate dispensation of study product, and scheduling of follow-up visits per protocol. QC/QA staff will monitor such indicators regularly and report outcomes to the investigator team. If any anomalies are noted, investigators will assess the root cause and support corrective and preventive actions. If the interim safety analysis reveals statistically significant differences of public health importance in safety outcomes, an investigator meeting will be convened to review the outcomes and determine safety of study continuation. In the event that other unforeseeable occurrences prevent study completion, investigator will review the circumstances, report to approving agencies, and inform participants accordingly.

4.12 Participants Withdrawal/Discontinuation Criteria

Participants may withdraw their consent to participate in this study with or without giving a reason. This will not affect their care at participating site. Once a participant and/or the consenting adult caregiver withdraws consent, this event will be documents and the participant discontinued from the study. No additional questionnaire data or blood samples will be collected, and previously collected samples will be destroyed. Participants who fail to adhere to visit schedules without withdrawing consent will be maintained in the study until the end of the study.

Clinicians may also use their discretion to discontinue participants from the study. This may occur following a serious adverse event, or if a clinical or other event emerges that compromises participant continuation. Such events will be documented and discussed with participants, with appropriate referrals made for follow-on (out of study) care.

5. Laboratory Procedures

5.1 Lab Evaluations

Several laboratory evaluations (Table 4) will be conducted in this study in order to assess study outcomes as follows:

- **Assay for serum antibodies against HPV antigens**: A competitive Luminex IgG assay will be used to assess the presence of antibodies and antibody titers at baseline, month 6 (PLHIV only) and month 7 (all participants). Among PLHIV, additional analyses for immunogenicity and immune response will be conducted using paper-based HPV-reactive IgG and multiplex ELISA for IFNg, TNF, IL2, CXCL10, granzyme, perforin, PTX3 at baseline and M7 IgG.

- **CD4 number and percent:** CD4+ lymphocyte values will be assessed at Month 0 and Month 7. For participants who have a current CD4+ lymphocyte result, this data will be abstracted from existing medical records. A current result is defined as a result from a test conducted within 30 days of study visit. For participants who do not have a current CD4+ result, testing will be conducted by the study.
- **HIV viral load:** HIV viral load values will be assessed at Month 0 and Month 7. For participants who have a current HIV viral load result, this data will be abstracted from existing medical records. A current result is defined as a result from a test conducted within 30 days of study visit. For participants who do not have a current HIV viral load result, testing will be conducted by the study.
- **Pregnancy testing:** Urine β-HCG levels will be tested using rapid testing on site prior to each vaccination. Individuals who have a positive test will have vaccinations deferred (see Section 4.10).

5.2 Conduct of Lab Evaluations

The laboratory evaluations will generally be conducted on site or at the Eswatini Prevention Center laboratories. The competitive Luminex IgG assay will be conducted at a protocol designated laboratory: Q2 Solutions LLC laboratory in Durham, North Carolina, USA. A material transfer information form (Appendix 9) is attached to this protocol and a material transfer agreement will be submitted after IRB approval is received. As this is a collaborative protocol between Baylor and ICAP at Columbia University, shared responsibilities have been assigned for the laboratory procedures. Following completion of Month 7 procedures, samples will be shipped on behalf of Ministry of Health to Baylor center of excellence for additional assays under this protocol and long-

term storage. In cases where available, current results from HIV viral load and CD4+ lymphocyte testing will be abstracted from existing medical records for participants.

Table 4: Laboratory Evaluations

	Location	Sample	Volume	Responsible	Baseline	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
PLHIV																	
Vaccinations: Two-dose regimen	Site		-		×						×						×
Lab Evaluations																	
Competitive Luminex IgG	MERCK	Blood (Plain)	10mL	ICAP	✓						✓	✓					
Urine Pregnancy Test	Site	Urine	10mL	ICAP & BCMCF	✓						✓						✓
CD4 cell count and percentage	Site/EHLS	Blood (EDTA)	5mL	ICAP & BCMCF	✓							✓					
HIV RNA (Viral Load)	Site/EHLS	Blood (EDTA)	5mL	ICAP & BCMCF	✓							✓					
Paper-based HPV reactive IgG	Baylor	Plasma	10µL	BCMCF	✓							✓					
Immunology	Baylor	Plasma	10µL	BCMCF	✓							✓					
HIV UNINFECTED WOMEN																	
Vaccinations: Three-dose regimen	Site		-		×		×				×						
Laboratory Evaluations																	
Competitive Luminex IgG	MERCK	Blood (Plain)	10mL	ICAP	✓							✓					
Urine Pregnancy Test	Site	Urine	10mL	ICAP & BCMCF	✓		✓				✓						
HIV Testing	Site/EHLS	Blood (EDTA)	1mL	ICAP & BCMCF	✓							✓					
Paper-based HPV reactive IgG	Baylor	Plasma	10µL	BCMCF	✓							✓					
Immunology	Baylor	Plasma	10µL	BCMCF	✓							✓					

5.3 Return of Laboratory Results

Clinically actionable results, such as pregnancy test results, CD4, HIV viral load will be shared with participants and placed in the participant's routine medical record. In some cases, CD4 and viral load data will be abstracted and would have been shared with participants during routine care. As all study participants will eventually get 3 doses of the 9vHPV vaccine, antibody test results are not clinically actionable. Antibody testing will be batched for efficiency and results will not be returned to participants.

5.4 Long term specimen storage and other use of specimens

Ministry of Health Eswatini has recommended that left-over blood will be shipped to the Baylor Centre of Excellence laboratory for long-term storage. Samples will be shipped to Baylor following completion of Month 7 primary immunogenicity analyses. At Baylor, samples will be stored on behalf of Ministry of indefinitely and used for serum antibody testing (paper based HPV-reactive IgG, Immunology testing (multiplex ELISA) as well as future use consistent with this protocol and

the informed consent. The Ministry of Health will still have full access to these samples while in storage at Baylor center of excellence.

Only samples for participants consenting for long-term storage will be archived. Samples belonging to participants who did not consent to long-term storage will be destroyed at the Eswatini CRS following completion of the Month 7 immunogenicity procedures. Should researchers wish to use such leftover blood samples for future studies, they will be required to submit a separate protocol describing the study and proposed use of specimens. The protocol will be submitted for approval to the respective regulatory and approving agencies in Eswatini (Eswatini Health and Human Research Review Board, Medicines Regulatory Unit) and internationally.

Specimens for Competitive Luminex 1Gg testing from will be shipped to the Merck designated laboratory for testing. Left over blood specimens for Competitive Luminex 1gG testing will be destroyed according to Merck policies and procedures and this destruction will be documented in the biorepository database.

6. Contraindications, Concomitant Medications and Adverse Events

6.1 Contraindications

Gardasil 9 is contraindicated in individuals with hypersensitivity, including individuals with severe allergic reactions to yeast (a vaccine component), or those who experienced a severe allergic reaction after a previous dose of GARDASIL 9.

6.2 Concomitant medications

Prior to each vaccination, participants will be assessed for new use of corticosteroids (prednisolone, hydrocortisone, dexamethasone etc.) in the past 2 weeks; other non-study vaccinations in the past 14-21 days and receipt of blood or blood products in the past 6 months. Information will be documented on the HPV Vaccination form (Appendix 5). Appropriate steps will be taken to delay vaccination for any participant who reports new use or receipt of any of these products.

6.3 Precautionary and Prohibited medications

Per the package insert, immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses),

may reduce the immune responses to vaccines. Use of these drugs is an exclusion criterion, and new use of these drugs will be assessed prior to each vaccination for all enrolled participants, and clinicians will determine whether vaccination may continue or not.

6.1 Adverse Event Reporting

Self-reported AEs will be collected and SAEs actively solicited by trained study staff. An adverse event is any untoward medical occurrence in a patient of clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Study staff will obtain details of AEs and SAEs through telephone follow-up calls after each vaccination. All information from these calls will be documented on the Adverse Event Form (Appendix 8). Additional telephone calls to participants and/or consultation of routine medical records may be required to follow-up details of AEs/SAEs, including date of resolution. In addition to follow-up telephone calls, participants will be counselled to actively report AEs at any point during the study by calling designated study team members, or during clinic visits. AEs and SAEs will be assessed through Month 7.

Grading Adverse Events: The study team will include clinicians (nurses and doctors) who will evaluate all reported adverse events. DAIDS AE grading tables [35] will be used to grade AEs in a standardized manner.

All grade 2 or greater AEs will be assessed for intensity, seriousness, and likely relationship to 9vHPV vaccination using an adverse event assessment job aid.

Serious Adverse Events: A serious adverse event is any adverse event occurring at any dose or during any use of the 9vHPV vaccine that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;

- Is another important medical event,

These events will be reported to the Eswatini Medicines Regulatory Unit (MRU) within 5 business days of the PIs becoming aware, and also reported to the Institutional Review Boards approving the protocol.

7. Data Management and Analysis

7.1 Data Collection

Data will be collected electronically using tablets at the study site. Eligibility checklists, questionnaire information, vaccination forms and other source documents will be captured electronically, with hard copies only allowable in exceptional circumstances e.g. tablet malfunction. From the tablets, data will be transmitted to a central database for additional cleaning and analysis. Where available, laboratory information system (LIS) data will be accessed for laboratory outcomes e.g. viral load. LIS data will be extracted and merged with the patient-level database for a complete analysis.

7.2 Assignment of PTIDs and the Study Link Log

In order to ensure confidentiality, data will be collected using a unique patient identifier (PTID). The PTID will be assigned at the time of eligibility and used on all study documents including screening and data collection forms, as well as the lab samples. To ensure appropriate linkage of study data to documents with participant information such as the informed consent form and participant contact information form (appendix 11), a study link log will be maintained. The link log will include the PTID and personal identifiers such as names, national ID number, electronic medical record number, laboratory information system number, date of birth, and ART/HIV clinic numbers. The link log will be maintained separately to ensure accurate linkage for follow-up activities and will not be included in the analytic dataset. The link will be destroyed after the study report is completed.

7.3 Data Management and Data Security

Data will be entered by study staff into password-protected electronic encrypted tablets using REDCap databases which will be developed for the study. REDCap is a secure web-based platform that was selected based on its appropriateness for mobile data collection and because it is certified

by Columbia University Irving Medical Center Information Technology as a multi-user platform for storing data. Data from the tablets will be uploaded daily to a secure server only accessible to the research team. No participant names or medical record numbers will be entered into the databases and all consent forms will be stored at the study site separately from any participant data files to protect confidentiality.

The data will be accessible to all principal investigators through a secured log-in access. The data team will periodically conduct quality review checks and queries will be sent back to the field team for resolution. Following resolution of all queries, a clean dataset will be prepared for analysis.

Data security measures will include human, physical and electronic procedures and protections during data collection, transfer and use:

- All electronic devices and databases will be encrypted and password protected, with access only provided to designated study staff. Data in transit will be encrypted with HTTPS/SSL.
- Electronic devices will only be used to capture and temporarily hold data, with upload to the study database encouraged as soon as data collection is complete.
- Data stored temporarily on electronic devices will be erased after successful upload to the study database.
- Electronic devices will be physically secured in locked cabinets at study sites when not in use to promote security.
- All paper-based data collection forms will be shredded after verification of successful upload to the study database.

Data will be stored on a secure server. Backups of the data will be exported from the cloud-based storage system onto a Columbia University secure server. All staff with access to data will receive training in data security and confidentiality and must sign a confidentiality agreement that affirms that they will abide by data security and confidentiality principles and procedures (Appendix 12). Although every effort will be made to keep participant information confidential, complete confidentiality cannot be guaranteed.

7.4 Statistical analysis

7.4.1 Analytic plan for primary and secondary objectives.

The primary objective of this analysis is to assess for non-inferiority in 7-month GMT between HIV-uninfected young women (15-26 years old) receiving the three-dose regimen vs each of three PLHIV groups (male 9-14 years, female 9-14 years, female 15-26 years) receiving the two-dose regimen. The primary non-inferiority hypothesis will be assessed by estimating a 95% confidence interval for the ratio of 7-month GMT between the three-dose group and, separately, each of the two-dose groups. Statistical assessment of non-inferiority is established if the lower bound of the 95% confidence interval for the GMT ratio is greater than 0.5.

The secondary safety profile objective will be described among all participants receiving at least one study vaccination who have follow-up data to assess safety. Serious adverse experiences, and other new medical conditions not present at baseline and reported as adverse experiences will be collected throughout the study. No formal hypothesis test will be conducted on safety.

The secondary completion objective will be compared by assessing the proportion of each pre-specified enrollment group successfully completing all scheduled vaccines.

7.4.2 Power calculations

This study's power calculation was based on the primary endpoint of immunogenicity (GMT) assessed at 7 months (or 4-weeks after the 6-month vaccination). A one-sided 2.5% significance level was used for the calculations. The power and sample size are calculated based on assumptions found in similar HPV vaccine protocols [36]: 1) the exclusion rates for the per protocol immunogenicity population are approximately 20% for the two-dose and 30% for the three-dose regimen, 2) the standard deviation of the natural-log-transformed titers of 1.2, and 3) non-inferiority margin for GMT ratio is two-fold (resulting in a lower 95% confidence limit bounded by 0.50). The estimates of exclusion rates and standard deviation are based on data from previous HPV vaccine studies and adjusted for potential missing data, and HIV sero-conversion among HIV uninfected women. Based on a sample size of 350 participants in each of four pre-specified groups, this study will have greater than 99.9% power to show non-inferiority to young adult PLHIV receiving the three dose regimens, assuming the true GMT ratio ranges from 0.95 to 1.2 (Table 5).

Table 5: Power Calculations

Sample size each group	exclusion rate + LTF%	final sample size	Non-inferiority Margin	True ratio	Power (%)
350	30%	245	0.5	0.95	>99.9
350	30%	245	0.5	1.0	>99.9
350	30%	245	0.5	1.2	>99.9
350	25%	263	0.5	0.95	>99.9
350	25%	263	0.5	1.0	>99.9
350	25%	263	0.5	1.2	>99.9

Note: PASS 14 was used for the sample size and power estimate.

- **Immunogenicity:** For 9vHPV, cLIA titers above or below the values designated by the Merck testing laboratory will be assigned for HPV subtypes tested as seropositive or seronegative. Seroconversion will be defined as change from seronegative status at baseline to seropositive at month 7 (Phase 1) and 12M dose (Phase 2). Geometric mean titers (GMT)s will be summarized by treatment group, and the ratios of mean GMTs between treatment and control and their 95% confidence intervals calculated around point estimates.
- **Adverse Events:** Grading of adverse events occurring within 14 days of each vaccination will be completed. All grade 2 or greater AE's will be recorded and assessed for potential relationship to vaccine administration. The number and percentage of participants experiencing SAE will be used to describe the safety profile of the two-dose and three-dose regimens in the study population.
- **Vaccine Series Completion:** The number and percentage of eligible participants completing 0, 2, 6 month vaccinations (according to regimen) will be calculated.

7.4.3 Descriptive statistics

Demographic and clinical characteristics of study participants will be summarized using the appropriate descriptive statistics for continuous and categorical variables and will include parameters for standard errors (SEs), and 95% confidence intervals (CIs). Descriptive statistics will also be used to quantify study participation, number of visits completed, and vaccines received, and adverse events experienced.

7.4.4 Bivariate analysis

Differences in the endpoints described in Table 2 by categorical variables such as age and sex and clinical status will be assessed, using the chi square ($\chi 2$) test. Odds ratios (unadjusted) and 95%

CIs for potential risk factors associated with the respective endpoints will be estimated using logistic regression models. Bivariate statistics will be used to compare PLHIV receiving 2-doses of 9vHPV vaccine to HIV negative women receiving 3-doses on the key outcomes of immunogenicity, safety and uptake.

7.4.5 Sensitivity Analysis

These will be conducted to examine nuances in the data, e.g. adolescents who transition from the 9-14 years age category to the 15-26 years age category before the second vaccine dose is administered

7.5 Data ownership and archival

All data collected through this protocol are the property of the Eswatini Ministry of Health and will be monitored by the study principal investigators. All principal investigators will have access to the database during data collection and de-identified datasets for analysis. Merck will remit the immunogenicity data conducted at their designated laboratory for merging with the overall study database.

The HPV study principal investigators have ultimate and equal responsibility to ensure data is protected during and after study implementation. At the completion of the study, each PI institution will receive a copy of the de-identified dataset for additional secondary analyses, and a copy will be deposited with the Ministry of Health's designated repository at the National Cancer Control Unit (NCCU). The de-identified data set will be stored indefinitely.

Other Co-investigators and other individuals wishing to access the data for the purpose of secondary data analysis should make a request in writing to the HPV study principal investigators. No data will be released without written consent of all principal investigator. Should researchers wish to use the de-identified data set for future studies, they will be required to submit a data request describing proposed use of study data.

8. Human Subjects Protections

8.1 Institutional Review Boards/Ethics Committees

The study protocol and associated materials will be reviewed and approved by the Eswatini Health and Human Research Review Board, Columbia University Irving Medical Center Institutional Review Board (CUMC IRB), the Eswatini Medicines Regulatory Unit and Baylor Eswatini IRB and the Baylor College of Medicine Biomedical Research and Assurance Information Network (BRAIN). Subsequent to the initial review and approval, the responsible IRBs/ECs will review the protocol at least annually.

8.2 Informed Consent/Assent Process

All potential participants will be asked to provide written informed consent/assent after provision of information regarding the study, its design, and potential risks and benefits. The study team will introduce the study using the assent/consent form and answer any questions the potential participant may have. If the potential participant is willing to participate, the study team will obtain written informed assent/consent and screen for study eligibility using a form developed for the study. Study assent and consent will be age-specific as follows:

- **Participants 18 years and older:** Prior to being enrolled in this study, all participants 18 years or older will be requested to give written informed consent.
- **Participants 15-17 years:** For all participants under 18 years of age, we will follow local precedents related to healthcare and research involving reproductive health services. In Eswatini, adolescents 12 years and older can independently seek routine health services and consent to both outpatient routine service delivery procedures such as HIV testing and sexual and reproductive health services, including family planning services [36, 37]. Based on this precedent, only adolescent assent will be sought for study participation. Obtaining parental/guardian consent for this age-group would be a challenge due to:
    - Some adolescents will be orphaned or no longer residing with parents/guardians and may already be seeking healthcare independently.
    - Discussion of vaccinations related to sexual health with parents and guardians may put some adolescents at risk of repercussion from parents/legal guardians, if access to such services is deemed to be associated with sexual debut or promiscuity.

- Discussion of eligibility criteria may lead to inadvertent disclosure of HIV status, should parents/guardians be previously unaware of this information.

We are therefore requesting a waiver of parental consent for participants who are 15 to 17 years of age based on 45 CFR 46.408(c) because 1) obtaining parental/guardian consent would not be a reasonable requirement and could put adolescent participants at risk, 2) appropriate mechanisms have been put in place by requesting the adolescents' assent and instituting data protection measures, and 3) this request is not inconsistent with Ministry of Health guidelines. Eswatini Ministry of health guidelines relating to access to HIV testing and sexual and reproductive health services allow adolescents 12 years and older to independently access such services. We have utilized a simple informed assent form which will be translated for better understanding of the study details, and these adolescents and young people are expected to independently volunteer for study participation. Additionally, this waiver does not adversely affect the welfare and rights of the participant, the study cannot practicably be implemented without the waiver, and participants will be provided sufficient information about the study and their participation.

- **Participants 9-14 years:** Both participant assent and parental/guardian permission or consent will be sought for these participants. Both the participant and the parent/guardian must agree to study participation for these children and adolescents to be enrolled. Should either the potential participant or the parent/guardian declines participation, this potential participant will be considered ineligible for the study.

All consent forms will be translated by native Siswati speakers from English into Siswati, and back translated to ensure accuracy of translations. Signed documentation of informed consent will be taken from all participants using paper forms. Participants will sign an additional paper copy of the consent form so that they can take it home. Trained interviewers will use standardized scripts for the informed consent process (Appendices 2a-2d). Following IRB guidelines, participants and parents/legal guardians who are unable to write their name and/or provide their signature will be asked to provide a thumbprint on the consent form. During the consent/assent process, we will emphasize to participants that: 1) participation is entirely voluntary, and the choice to participate or

not will in no way influence the quality of medical care they receive; and 2) participants may leave the study at any time for any reason without compromising the quality of health care received.

8.3 Potential Risks

The potential risks to participants in the study include:

Risks from 9vHPV vaccine: According to the HPV vaccine manufacturers, the most common adverse events associated with 9vHPV vaccination include injection pain, swelling, redness, stinging, bruising, bleeding at the injection site, and headache, fever, nausea, diarrhea, abdominal pain, and syncope [31, 38, 39]. Such anticipated risks will be included in the informed consent/assent forms. Throughout the study, investigators will collect data on experience of adverse events, and will quantify, qualify, document, and report these events as described in section 4.8 above.

Breach of confidentiality: While all efforts will be made to protect participant confidentiality as described in 5.2 above, the risk of breach of confidentiality remains. Participants and parents/legal guardians will be made aware of this potential risk during the informed consent process. Study staff will receive training on procedures to protect participant confidentiality. All study staff will sign a confidentiality form pledging not to disclose protected health information to other persons or entities.

8.4 Potential Benefits

Participants are expected to benefit from the immunity conferred by the 9vHPV vaccine, which is expected to lead to protection from most HPV infections and reduce the risk of developing cervical and penile cancer. Communally, this would lead to fewer people infected with HPV over time and is expected to lead to reduced onward transmission of HPV.

8.5 Confidentiality

Study information with personal identifying information (PII) will be physically and electronically separated to protect participant confidentiality. Patient names and other identifiers will not be collected included in the analytic dataset, and the link long that links PII to outcome data will be separately encrypted. All study staff with access to data will receive training in data security and confidentiality and must sign a confidentiality agreement that affirms that they will abide by data

security and confidentiality principles of the study and not disclose protected health information to other persons or entities.

8.6 Social Harms

In addition to clinical events, details of any social harms that may occur during the study will also be passively captured. Details of any reported social harms, including stigmatization, verbal, physical and sexual abuse, etc, will be captured using a social harms form in Appendix 10 and evaluated for seriousness and relatedness using the same criteria as above. Should social harms meet the criteria of an SAE, they will be reported accordingly.

8.7 Compensation

Participants may be offered refreshments during study visits that involve more procedures e.g. screening/enrolment, or if a visit is prolonged due to AE assessment. Study visits will be scheduled around existing routine visits, to avoid extra inconvenience to participants. Transport costs for study related visits occurring outside existing routine visits will generally be reimbursed at a rate in-line with public transportation using the reimbursement according to the study standard operating procedures. Participants who miss visits will be called or visited at home according to Ministry of Health guidelines, to support their return to clinic.

8.8 Data Safety Monitoring

As this is not a clinical trial with an investigational product and safety data are already available [31, 40], investigators do not anticipate safety issues that would require oversight by a data safety monitoring board. Each serious adverse event will be reviewed by the study investigators and reported to all approving agencies within 5 days of occurrence. Investigators will statistically assess comparative safety outcomes under secondary objective 1. An interim safety analysis will be conducted after all participants have received their first dose of the vaccine. If this interim analysis reveals statistically significant differences of public health importance in safety outcomes, and investigator meeting will be conducted to review the outcomes and determine safety of study continuation.

In addition to reporting serious adverse events to EHHRRB, BRAIN, CU IRB and MRU within 5 days, these events will also be reported to the Eswatini Ministry of Health Expanded Programme on

Immunization (EPI) in the same time period. EPI is a Ministry of Health entity, that works closely with MoH pharmacovigilance unit and provides oversight for all immunization and vaccination activities in Eswatini.

All AEs which are grade 3 or higher and those not meeting an expedited adverse event criteria will be submitted to EHHRRB, BRAIN, CU IRB, MRU and EPI during submission of progress reports or at annual renewal. A full report of AEs will be included in the final study report.

9. Timeline and implementation during the SARS-CoV-2 era

As this is a facility-based study, all standard MOH infection prevention measures will be applied during study implementation. This will include (but is not limited to)

- Use of appropriate personal protective equipment (PPE) by study staff such as masks, gloves and other coverings,
- Wearing of face masks by participant,
- Routine symptom screening at the designated triage areas in the health facility,
- Social distancing during participant interactions,
- Hand hygiene.

The anticipated timeline for this study is shown in Table 6 below.

Table 6. Study Timeline

	2021				2022				2023			
	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec
Protocol development	X											
Ethical submissions		X										
Ethical approvals			X	X								
Screening and enrolment					X	X	X					
Follow-up					X	X	X	X	X	X	X	
Data cleaning and analysis							X	X	X	X	X	X
Interim safety analysis							X	X				
Report writing										X	X	X
Dissemination											X	X

10. Budget

Sufficient funding to support staffing and implementation of study activities has been allocated for the entire study duration. This study is supported by the United States President's Emergency Plan for AIDS Relief (PEPFAR), through the United States Agency for International Development funded Reaching Impact, Saturation, and Epidemic Control (RISE) program, under the terms of the cooperative agreement 7200AA19CA00003. Study-related activities conducted by Baylor staff are supported by Bristol Myers Squib Foundation funding to Baylor College of Medicine Children's Foundation Eswatini. Study vaccines will be supplied by MERCK.

11. Dissemination & Publication Plan

11.1 Dissemination Plan

Overall dissemination of the study results for this study will be coordinated by the principal investigators in consultation with the Ministry of Health and study sponsors. Local study dissemination activities will be led by MOH, and guided by the sponsor and investigator teams. Dissemination will include in-person or virtual dissemination meetings, a study report, conference presentations, and peer-reviewed publications.

Table 7: Illustrative Study Results Dissemination Plan

Activity	Target Audience	Responsible persons
1. Principal investigator review of study results	Principal Investigators	All PIs
2. Inform MoH leadership and Senior management, as well as Senior Managers of Implementing Partners	• Core Leadership Group • MOH Senior Managers • Country Directors • UN, WHO, UNICEF Representatives	Local PIs
3. Inform Study Team	• MOH Stakeholders • ICAP at Columbia University • Baylor Foundation Eswatini Team	All PIs
4. Inform technical stakeholders	• Technical working groups (TWGs) for Cancer, HIV, EPI, HPV • Study Advisory Groups • MOH National Health Research and Innovation Department	Local PIs Study Team Leads
5. Submission of study report to IRBs	• EHHRRB • MRU	All PIs

Activity	Target Audience	Responsible persons
	• BRAIN • CU IRB	
6. Workshop to develop and review study recommendations	MOH Technical stakeholders	National Cancer Control Unit
7. Dissemination to regions and health facilities and	RHMT Health Facility Managers	National Cancer Control Unit

11.2 Publication Plan

The protocol team will publish study outcomes in a study report, and in peer reviewed journals. Authorship of published products will be consistent with standard ICJME criteria for author contribution, to reflect significant contribution to the design and conduct of the trial, performance or interpretation of the analysis, and/or writing of the publication. Collaborators who do not meet authorship criteria will be acknowledged accordingly.

11.3 Post study advocacy

Depending on the results of the study, HPV vaccine investigators will work with the Eswatini National Cancer Control unit and MoH Expanded Program for Immunizations for a review of National guidelines. These guidelines will inform the nationwide HPV vaccination program in Eswatini.

12. Appendices

Appendix 1: Vaccine information sheet for information, education and communication (IEC)
Appendix 2a: Eligibility checklist for PLHIV 9-26 years
Appendix 2b: Eligibility checklist for HIV uninfected women 15-26 years
Appendix 3a: Screening and enrolment assent form_PLHIV_ 9-14 years
Appendix 3b: Screening and enrolment caregiver permission for PLHIV_9-14 years
Appendix 3c: Screening and enrolment assent consent form_PLHIV_15-26 years
Appendix 3d: Screening and enrolment assent consent form_HIV negative women_15-26 years
Appendix 4: Study questionnaires
Appendix 5: HPV vaccine form
Appendix 6: Vaccination and appointment card
Appendix 7: Data abstraction form

Appendix 8: Adverse event form
Appendix 9: Material transfer information form
Appendix 10: Social harm form
Appendix 11: Contact information form
Appendix 12: HPV Study confidentiality agreement
Appendix 13: Media recruitment script

13. References

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