A randomized, placebo-controlled trial of pre-treatment HPV vaccination on outcomes to LEEP treatment of cervical high grade squamous intraepithelial lesions in HIV-infected women.

Industry Support Provided by:
VERSION 1.0
8 November, 2014
Includes LOA #1
MHAVE Study

Merck & Co., Inc.
MSD # 50971
#V501-190

MERCK VACCINE STUDY VERSION 1.0 (LOA 1)
MHAVE STUDY
3 November 2014

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GLOSSARY OF PROTOCOL-SPECIFIC TERMS

ASC-H	atypical squamous cells, cannot exclude HSIL
ASCUS	atypical squamous cells of undetermined significance
AIS	adenocarcinoma in situ
CIN	cervical intraepithelial neoplasia
HPV	human papillomavirus
HSIL	high grade squamous intraepithelial lesion
LSIL	low grade squamous intraepithelial lesion
LEEP/LLETZ	loop electrical excision procedure/large loop electrical removal of transformation zone
PPA	per-protocol analysis
qHPV	quadrivalent HPV vaccine
SUSAR	suspected, unexpected serious adverse reactions
VAERS	Vaccine Adverse Event Reporting System
VaIN	vaginal intraepithelial neoplasia
VIN	vulvar intraepithelial neoplasia
VLP	virus-like particle

SCHEMA

A randomized, placebo-controlled trial of pre-treatment HPV vaccination on outcomes to LEEP/LLETZ treatment of cervical high grade squamous intraepithelial lesions in HIV-infected women.

DESIGN	This is a single-center, randomized, double-blinded, placebo-controlled, phase II trial of the quadrivalent human papillomavirus vaccine (qHPV) in HIV-infected women to prevent occurrence of cervical HSIL after LEEP/LLETZ. At entry and prior to vaccination, participants will undergo colposcopy with directed biopsies, cervical cytology, and stored HPV testing. Participants will then be randomized to the quadrivalent vaccine or saline placebo to be given at entry, week 4, and week 26. Women will have LEEP treatment at week 4. During follow-up, participants will be seen for cervical cytology, colposcopy with directed biopsies at weeks 26 and 52, and HPV specimens collection. Treatment assignment will be unblinded after study follow-up is completed for the last study participant. Women aged 45 or less randomized to placebo will be offered open label HPV vaccine. A subset of women (n=120) will participate in an immunology sub-study. These women must have a CD4 count >200 cells/mm^3 and a plasma HIV-1 RNA of <200 copies/mL. These women will undergo cervical fluid and blood collection, for assessment of gene expression signatures, HPV antibodies, and markers for immune activation.
DURATION	Each participant will be followed for 1 year
SAMPLE SIZE	180 participants
POPULATION	HIV-1-infected women, aged 18 years or older, with cervical HSIL
STRATIFICATION	Participation in immunology subset
REGIMEN	qHPV vaccine or placebo 0.5 mL at entry, week 4, and week 26.

1.0 HYPOTHESIS AND STUDY OBJECTIVES

1.1 Hypothesis

HPV vaccination will reduce the occurrence of cervical HSIL post-LEEP/LLETZ.

1.2 Primary Objective

To determine the effect of pre-treatment quadrivalent HPV vaccination on outcomes to LEEP/LLETZ treatment of cervical HSIL on persistent and/or recurrent HSIL among HIV-infected women

1.3 Secondary Objectives

1.3.1 To compare cervical cytology abnormalities, and squamous intraepithelial lesions on cervical biopsy between arms at week 26 and 52.

1.3.2 To store cervical swabs for high-risk HPV testing to evaluate persistent, recurrent, and incident HPV infections.

1.3.3 To explore vaccine efficacy in different subgroups: women with cervical HSIL confirmed on LEEP/LLETZ specimen at week 4, and women with positive margins on LEEP/LLETZ specimen at week 4.

1.3.4 To collect samples for immune sub-study for assessment of gene expression signatures, HPV antibodies, and markers for immune activation associated with primary objective outcomes.

2.0 INTRODUCTION

2.1 Background

Cervical cancer is one of the most common cancers in women. The most recent compilation of global data indicates that an estimated 490,000 new cases of cervical cancer occur annually among women worldwide and nearly 80% of these are in developing countries, where screening programs are not well established. It is estimated that 270 000 women die annually from cervical cancer, 85% of these in low resource nations[1-3]. In Africa, cervical cancer makes up 23.3% of all cancers in women[4]. HIV-infected women have high rates of cervical intraepithelial neoplasia (CIN) especially in resource limited areas. South Africa and Zambia report that high grade squamous intraepithelial lesions (HSIL) were found in approximately 30% of HIV-infected women [5, 6]. The Women's Interagency HIV Study (WIHS), a US cohort, found that HIV-infected women had a CIN prevalence of 16%, of which 87% were low grade squamous intraepithelial lesions (LSIL) and 13 % (HSIL), compared to only 4 % in HIV-uninfected women[7]. With

improved access to ART, HIV-infected women are living longer allowing time for persistent high-risk HPV infections and HSIL to progress to invasive cancer.

Cervical HSIL (cervical intraepithelial neoplasia grade 2 or 3), the precursor lesion to cervical cancer, is treated by either ablative or excisional methods. A very efficient and effective outpatient procedure requiring only local anesthesia is LEEP/LLETZ (loop electrosurgical excision procedure/Large Loop Excision of the Transformation Zone). LEEP/LLETZ uses a thin electrified wire to remove the lesion up to 7 mm in depth from the transitional zone. Treatment failure rates (as defined as incomplete ectocervical and/or endocervical margins on pathology specimens) are between 10-15% for the LEEP/LLETZ procedure in immunocompetent women [8]. The LEEP/LLETZ failure rate among HIV-infected women is up to a 50% failure rate worldwide. The incomplete excisional margin significantly increases the risk of persistence and/or recurrence of disease. The incomplete excisional lesions are thought to be due to the greater size of the HSIL lesions and the multifocal nature of these lesions in HIV seropositive women[9-12]. Additional studies suggest that a combination of cytology and high risk HPV testing 6 months post-LEEP/LLETZ are highly predictive of persistent/recurrent HSIL[13,14] . Other factors that may be associated with increased risk of recurrence and /or persistence of disease are persistent/recurrent HPV infection, CD4 count <200 cells/mm^3, plasma HIV-1 RNA> 50 copies/ml and not receiving ART [9-12, 15-17].

Persistent HPV infection

Persistent and/or recurrent HPV infection is a risk factor for refractory disease [15, 16]. However few studies have investigated the effect of LEEP/LLETZ treatments on HPV clearance in HIV-infected women. Massad and colleagues reported on 170 HIV-infected women from the WIHS (Women's Interagency HIV Study) and HERS (HIV Epidemiology Research Study) cohorts who underwent cervical treatment for CIN. They found that HPV commonly persisted among women who had abnormal cervical cytology after treatment[17]. More data are available for HIV-uninfected women. Elgren and colleagues reported retrospectively on 109 women treated for CIN with either cervical conization or cryotherapy[18]. Eighty-four (81%) were positive for HPV. Thirty-one women were treated with cervical cryotherapy and 15 (48%) were positive for the same type 3 months later. Persistence of HPV was approximately 20% 12 months after cervical cryotherapy. Of note, HPV clearance was greater for women treated with cervical conisation [18]. A5282 (an ACTG international multisite test and treat study) will provide important data on clearance of HPV in HIV-infected women after cryotherapy and LEEP/LLETZ.

HIV-infected women commonly require chronic treatment of cervical dysplasia (i.e. multiple LEEP/LLETZs, cone biopsies, and/or hysterectomy). These repeated procedures increase the morbidity for the patients in terms of increase risk of cervical incompetence and adds to the expense and patient load in health care systems especially in resource limited countries where the prevalence of high grade is so high. New strategies are needed to improve outcomes to treatment of high grade CIN.

HPV Vaccination in HIV-infected populations

Gardasil (quadrivalent HPV vaccine consisting of recombinant L1 particles for types 6, 11, 16 and 18) has been shown to prevent cervical, vaginal, anal and external genital lesions caused

by the vaccine types [19]. However, it does not hasten clearance of prevalent HPV infections[20] nor prevent or treat lesions caused by ongoing infection with the vaccine types[21, 22].

ACTG A5246/AMC052 was a single-arm, open-label trial evaluating the safety and immunogenicity of the quadrivalent HPV vaccine (6, 11, 16, and 18) in 109 HIV-1-infected men without HGAIN [23]. The vaccine was found to be safe. There were no appreciable effects on plasma HIV-1 RNA or CD4+ counts. There were no grade 3 or 4 events that were possibly, probably, or definitely related to vaccination. The vaccine was highly immunogenic with seroconversion rates being 95% or greater for each of the four vaccine types. Among women, the immunogenicity, safety, and tolerability of the quadrivalent HPV recombinant vaccine directed against types 6, 11, 16, and 18 was assessed in the ACTG 5240 study. This was a phase II, open-label, single arm study with stratification by CD4+ cell count of 319 women aged 19 to 45 in the US, Brazil and South Africa. Among 222 women with CD4 counts over 200 and seronegative for each type at baseline, the vaccine was highly immunogenic with seroconversion rates ranging from 84 to 100% for each HPV type in the vaccine. There were no differences in immunogenicity rates between women with a CD4 count over 350 compared with counts between 200 and 350. There were no safety concerns related to the vaccine[24].

HPV Vaccination in the Context of Dysplasia Treatment

Joura and colleagues examined the women (HIV negative) receiving excisional cervical treatments while participating in the clinical studies of the quadrivalent HPV vaccine. In the combined retrospective evaluation of the two phase 3 HPV vaccine trials, 587 vaccine and 763 placebo recipients underwent treatments of the cervix[25]. Women who had received the quadrivalent HPV vaccine had a 46% lower risk of subsequent HPV disease and a 65% [95%CI: 20-86] lower risk of high grade CIN as compared to women who had received placebo. Interestingly, the observed effect was not restricted to infection or dysplasia caused by vaccine types.

Swedish and colleagues conducted a retrospective chart review of HIV-uninfected men who have sex with men who were diagnosed with high grade anal intraepithelial neoplasia (HGAIN). They showed that men who had been previously vaccinated had a 50% lower risk for recurrent or persistent HGAIN after undergoing ablative treatment of HGAIN [26].

These data suggest that HPV vaccination may improve outcomes to treatment of dysplasia. HPV infects the epithelium through microabrasions in the epithelium[27]. HPV antibodies induced by HPV vaccination, when present in high titers, prevent infection by binding the virus and preventing interaction with the acellular basement membrane[28]. At lower antibody titers, this interaction is not blocked. Lower antibody titers block the interaction of the HPV virion and the basal epithelial cells thereby preventing infection by a second mechanism[29]. This second mechanism is thought to be how HPV vaccines confer long lasting protection even when antibody titers have fallen to low levels.

Persistence of high risk HPV after LEEP/LLETZ is a major risk factor for recurrence of high grade CIN. LEEP/LLETZ may clear HPV infection of the cervix, but will not clear HPV infections at other sites in the genital tract (i.e. vagina or vulva) that may lead to re-infection of the healing cervix. Women are also at risk for re-infection from their sexual partners. We hypothesize that

HPV antibodies induced by HPV vaccination present in the cervical area after LEEP/LLETZ will protect the healing epithelium from re-infection with HPV by the two mechanisms discussed above.

There are limited data on the immunogenicity of the quadrivalent HPV vaccine 4 weeks after a single dose. Among seronegative young, HIV-uninfected women, the geometric anti-HPV16 titers 1 and 4 weeks after a single dose of the quadrivalent HPV vaccine were 30 [95% CI 18, 50] and 113 [80, 159], mM/mL[30] [unpublished data from Merck]. There are data 4 weeks after a single dose of Cervarix (bivalent HPV vaccine using the same viral like particles for HPV 16 and 18, but a different adjuvant). 99% and 98% of HIV-uninfected women age 15-25 seroconverted to type 16 and 18 respectively[31]. The Advisory Committee on Immunization Practices, which sets the standard for immunizations in the US, accepts a second HPV vaccination as early 28 days after the 1st vaccine.

HPV and immune regulation

The understanding of the immunoresponse of HPV infection and to the HPV vaccine is also very limited. A recently presented abstract at CROI by Papasavvas et al evaluated the systemic and local immune responses in HIV infected women with suppressed HIV viral loads on ART with and without HR HPV infection. Presence of HR HPV infection irrespective of lesion grade (CIN 1-2-3) was associated with increased T cell activation in otherwise ART suppressed HIV infected women with higher CD4 counts (median CD4 count approximately 500) Changes between CIN 1 and 3 did suggest greater levels of negative immunoregulatory molecules expression even if increased in all groups with HR HPV types as compared to women without HR HPV [32]. In addition, cervical biopsy analysis supports that changes in peripheral blood also relate to local tissue changes in gene expression as well as detectable gene expression changes with increasing cervical dysplasia. Further evaluation of immune activation, immunoregulation, gene expression and HPV immunity in ART suppressed women is required. It remains unknown how quickly the biological changes associated with HR HPV cervical dysplasia would reverse after a LEEP/LLETZ procedure with or without vaccine immunotherapy if targeted to target HR types. Note that in addition to the cervix, we will also monitor presence/absence of high-risk types in anal region[33-37], to control for the systemic impact of independent high-risk infection in the anus might have on the immune response before/after LEEP/LLETZ.

2.2 Rationale

Women with HIV have high rates of cervical HSIL, particularly in South Africa, and are at higher risk for persistent or recurrent HSIL after LEEP/LLETZ, which could increase their risk for progression to cervical cancer[38]. Strategies are needed to improve outcomes to cervical treatments in HIV-infected women. This is even more critical in resource constrained settings where cervical cancer screening may be very infrequent; follow-up is difficult and health care systems overwhelmed.

In South Africa, the quadrivalent HPV vaccine is indicated for the active immunisation in females 9 to 45 years of age for the prevention of cervical, vulva, vaginal and anal cancer, precancerous

or dysplastic lesions, genital warts and in males 9 to 26 years of age for the prevention of anal cancer, precancerous or dysplastic lesions, external genital lesions and infection caused by HPV types 6, 11, 16 and 18. The recommended age in girls is age 11-14. HPV vaccination is not routinely available in South Africa, especially for those aged 18 or over.

This proposal will evaluate whether these outcomes can be improved with HPV vaccination prior to cervical LEEP/LLETZ. While HPV vaccination is unlikely to hasten clearance of established type 16 or type 18 infections, it may prevent new infections or re-infection of the cervix after LEEP/LLETZ treatment and subsequent recurrent disease. If successful, this study will provide a proof-of-principle for this strategy. Next-generation vaccines protecting against additional types of high-risk HPV would lead to even greater benefits.

3.0 STUDY DESIGN

This is a single center, randomized, double-blinded, placebo-controlled, phase II trial. The study population will include 180 HIV-1-infected women, aged 18 years and older with cervical HSIL on biopsy.

Participants will be consented per SOP of the study unit. Cervical colposcopy with biopsy of visible lesions will be done to evaluate for cervical HSIL. This is only done if a result is not available within the last 90 days. Participants will undergo blood testing for CD4 and HIV viral load if not available within the last 90 days.

At entry, participants are randomized 1:1 to HPV vaccination or placebo. Participants will undergo cervical swabs for HPV DNA PCR. A pap smear is done if a result is not available within the last 180 days. Participants will receive vaccine or placebo at entry, week 4, and week 26.

At week 4, women will be seen for LEEP/LLETZ treatment of cervical HSIL. The LEEP/LLETZ is performed according to IARC or WHO recommendations at the discretion of the treating provider.

At week 26, and week 52, participants will be seen for cervical swabs for HPV testing and cervical cytology. Cervical colposcopy and biopsy of visible lesions will be performed. If no lesions are seen at week 26 and 52, then random biopsies will be done at 6 and 12 positions on the cervix. At least two biopsies should be obtained for all participants. Endocervical curettage can be performed at the discretion of the treating provider. Participants diagnosed with cervical HSIL at week 26 will be treated according to South African standard of care; options include a repeat LEEP/LLETZ, cone biopsy or other treatment as determined necessary by the local provider. These treatments are not specified by the study and do not constitute study visits. These women should remain in follow-up and be seen at week 52. Women diagnosed with cervical HSIL at week 52 will be seen for treatment as determined by South African standard of care. Other HPV related lesions, such as vaginal, vulvar or perianal HSIL, will be referred to appropriate specialist and treated per South African standard of care.. This is not determined by the study.

Participants will be told of their treatment assignment after the last participant has completed study follow-up. Participants age 45 or less who received placebo will be offered open-label HPV vaccine. Please note that this vaccine is approved for women up to age 45 in South Africa. This will be provided by the study and dispensed by the study pharmacy. No additional follow-up is planned for this open-label administration of HPV vaccine.

In addition, 120 participants will be asked to participate in the Immunology sub-study. Participants are eligible if their CD4 count is >200 cells/mm^3, and plasma HIV RNA level is <200 copies /ml. Participants in the immunology sub-study will give cervical fluid specimens using small sponges inserted in the cervical os for assessment of immune activation, extra blood specimens (i.e., PBMC for immunophenotypic characterization, plasma for HIV-1 RNA testing and serum for assessment of HPV antibodies and soluble markers of immune activation), at entry, week 4, weeks 26 and 52. The plasma HIV-1 RNA specimens will be stored for evaluation when funding is available using the Abbot HIV viral load platform. The PBMC, serum, , and cervical fluid specimens will be stored until funding is available for testing. This testing will be performed at the WISTAR Institute in Philadelphia, PA, USA. In addition, recut slides of the colposcopic biopsies of women participating in the immunology sub-study will be sent to Wistar Institute in Philadelphia, Pennsylvania for immunohistochemical evaluation of cervical cellular inflitrates. Anal swabs will be done tested for HR HPV typing. See Table below for description of specimens to be collected. The sub-study procedures will be done before the vaccination and any colposcope or LLETZ/LEEP procedures if at all possible.

Sample	Purpose	Lab	Participants to be notified of results	Stored until funding available or real time	MTA required
Cervical cyto broom	Assessment of dysplasia	NHLS	Yes	Real time	No
Cervical biopsies, LEEP/LLETZ specimens	Assessment of dysplasia	NHLS	Yes	Real time	No
Cervical Swab	HPV testing	UCT HPV lab	No	Stored	No
Anal swab	To determine HPV present and type in anal canal	NHLS	No	Stored	No
Cervical fluid	Cytokine/Chemokine profiling	Wistar	No	Stored	Yes
Plasma	HIV RNA	CLS Wits	Yes	Real time for screening	No

				/ stored for post-entry	
Blood	Rapid HIV testing, CD4 cell count	CLS Wits	Yes	Real time	Yes
Serum	HPV antibodies, soluble markers of immune activation	Wistar	No	Stored	Yes
PBMC	Flow cytometry for immunophenotyping	Wistar	No	Stored	Yes
Whole blood lysate	Gene expression profiling of pathways associated with HPV immune evasion	Wistar	No	Stored	Yes
Recut histology slides from Colposcopic biopsy	immunohistochemical evaluation of cervical cellular infiltrates	Wistar	No	Stored	Yes

4.0 SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

4.1.1 HIV infection documented by positive HIV test by two different criteria; either 2 different rapid HIV tests of different manufacturers, plasma HIV-1 RNA level >5,000 copies/mL, or Western blot).

4.1.2 Women aged ≥ 18 years.

4.1.3 Cervical HSIL on biopsy (i.e. CIN2 and/or CIN3)

4.1.4 For participants of reproductive potential, negative serum or urine pregnancy test with a sensitivity of <25 mU/mL within 48 hours prior to study entry.

NOTE: Reproductive potential is defined as women who have not been postmenopausal for at least 24 consecutive months (i.e., who have had menses within the preceding 24 months) or women who have not undergone surgical sterilization (e.g., hysterectomy, bilateral oophorectomy, tubal ligation, or salpingectomy).

4.1.5 Contraception requirements

All study participants must agree not to participate in a conception process (e.g., active attempt to become pregnant or in vitro fertilization) during study participation (from the time of study entry until week 52).

If participating in sexual activity that could lead to pregnancy, the study participant must agree to use at least one reliable form of contraceptive

from the time of study entry until week 52. At least one of the following contraceptives MUST be used appropriately:

- Condoms (male or female)
- Diaphragm
- IUD
- Hormone-based contraceptive

4.2 Exclusion Criteria

4.2.1 History or current biopsy diagnosis of invasive or microinvasive cervical, vaginal, vulvar or anal cancer

4.2.2 Prior hysterectomy

4.2.3 Cervical cryotherapy or LEEP/LEETZ within one year of entry.

4.2.4 Cervical, vulvar, or vaginal lesions suspicious for cancer, unless biopsies show no invasive cancer

4.2.5 Prior receipt of one or more doses of an HPV vaccine.

4.2.6 Receipt of anticoagulants other than aspirin or nonsteroidal anti-inflammatory drugs (NSAIDS) within 14 days prior to entry.

4.2.7 Known allergy/sensitivity or any hypersensitivity to yeast or any of the components of the study product or its formulation (see section 5.2 for a list of components).

4.2.8 Hemophilia or other bleeding diatheses.

4.2.9 Use of any systemic antineoplastic or immunomodulatory treatment, systemic corticosteroids, other than inhaled corticosteroids or prednisone ≤ 10 mg (or equivalent) , investigational vaccines, interleukins, interferons, growth factors, or intravenous immunoglobulin (IVIG) within 45 days prior to study entry.

NOTE: Routine standard-of-care vaccines (including hepatitis A, hepatitis B, influenza, pneumococcal, and tetanus vaccines) are not exclusionary.

4.2.10 Breastfeeding

4.2.11 Less than 3 months post-partum

4.3 Study Enrollment Procedures

4.3.1 Prior to implementation of this protocol, and any subsequent full version amendments, the protocol and the protocol consent form approved by the local ethics committee (EC) and MCC.

Once a candidate for study entry has been identified, details will be carefully discussed with the participant. The participant (or, when necessary, the legal guardian if the participant is under guardianship) will be asked to read and sign the approved protocol consent form.

4.3.2 Randomization

Participants will be randomized using permuted block randomization using block sizes of six, stratified by participation in the immunology substudy. The randomization list will be kept under lock and key in the investigational pharmacy.

4.4 Co enrolment Guidelines

Participants may be co-enroled in other observation clinical trials without consulting study chairs but will need ethics approval prior to co-enrollment. The study chairs should be notified of participation in any other clinical trials

5.0 STUDY TREATMENT

Study treatment is defined as:

- Quadrivalent human papillomavirus (types 6, 11, 16, and 18) recombinant vaccine (qHPV) or saline placebo.

- Loop electro excision procedure (LEEP), also known as large loop excision of the transformation zone (LLETZ)

Antiretroviral therapy (ART) will not be supplied by the study and should be continued or initiated per the local standard of care. ART must be obtained outside of this study.

5.1 Regimens, Administration, and Duration

5.1.1 Regimens

Participants will be randomized to receive the quadrivalent HPV (types 6, 11, 16, and 18) recombinant vaccine, 0.5 mL, or the placebo for qHPV at entry (day 0) and at weeks 4 and 26.

Please note: The recommended schedule for the quadrivalent HPV vaccine is 0, 2, and 6 months. This study is using a modified schedule where the second vaccine is given earlier. This alternative regimen is acceptable according to the Advisory Committee on Immunization Practices, which sets the standard for US immunization practices and preferred by MERCK for use in this study. This is the vaccination schedule for the bivalent HPV vaccine, which is very similar to the quadrivalent HPV vaccine. This minimizes the delay between diagnosis of HSIL and LEEP/LLETZ.

All participants will undergo LEEP/LLETZ at week 4.

All participants will be followed for 1 year post-randomization.

5.1.2 Study Product Administration

qHPV or saline placebo will be administered as three separate 0.5 mL doses according to the visit schedule. The vaccine is to be used as supplied; no dilution or reconstitution is necessary. The vaccine will be administered intramuscularly in the deltoid region of the upper arm or the higher anterolateral area of the thigh in the clinic by study personnel using aseptic technique during preparation and administration. All injections should preferably be given in the deltoid region and in the non-dominant arm, however if this is not feasible, the dominant arm may be used. The participant should be seated during vaccine injection.

The vaccine must not be injected intravascularly, subcutaneously, or intradermally.

Vaccination should be deferred at the discretion of the site investigator if the participant is febrile or acutely ill.

Following each vaccination, clinic staff will observe the participant for 15 minutes.

5.2 Study Product Formulation

5.2.1 Quadrivalent HPV Virus-Like Particle (VLP) Vaccine

Each 0.5 mL dose of qHPV (types 6, 11, 16, and 18) recombinant vaccine contains approximately:

• HPV 6 L1 protein	20 mcg
• HPV 11 L1 protein	40 mcg
• HPV 16 L1 protein	40 mcg
• HPV 18 L1 protein	20 mcg
• Amorphous aluminum hydroxyphosphate sulfate adjuvant	225 mcg
• Sodium chloride	9.56 mg
• L-histidine	0.78 mg
• Polysorbate 80	50 mcg
• Sodium borate	35 mcg
• Yeast protein	< 7 mcg
• Water	q.s. (‘quantity sufficient’)

5.2.3 Placebo for Quadrivalent HPV Vaccine

Sodium Chloride for Injection USP, 0.9% will be used as the placebo for qHPV vaccine.

For the purpose of this study and to maintain the blind of study product, the Sodium Chloride for Injection USP, 0.9% must be stored refrigerated at 2°C to 8°C (36°F to 46°F).

5.3 Study Product Preparation

5.3.1 Quadrivalent HPV (VLP) Vaccine

- One pre-filled syringe of qHPV will be used to prepare this dose. Prior to injection, the pharmacist will shake the vaccine syringe well before use. Thorough agitation immediately before administration is necessary to maintain suspension of the vaccine.
- qHPV vaccine should not be diluted or mixed with other vaccines.
- After thorough agitation, qHPV vaccine will be a white, cloudy liquid. Do not use the product if particulates are present or if it appears discolored.
- Using aseptic technique, the pharmacist must inject the 0.5-mL dose of vaccine from the pre-filled syringe to a sterile syringe using a needle through the orifice of sterile syringe.
- Once drawn into the syringe, the vaccine should be kept cold (2° to 8°C) until it is ready to be administered to the participant. Use promptly.
- The pharmacist will place an overlay on the syringe. With the overlay in place, the syringe should be labeled as *"Quadrivalent HPV Vaccine or Placebo"* to maintain blinding. The syringe should also be labeled with the date/time product was withdrawn from the refrigerated vial into the syringe and the participant's identifier.
- All used vaccines should be discarded appropriately.

5.4.2 Placebo for Quadrivalent HPV (VLP) Vaccine

- One vial of normal saline (labeled as Sodium Chloride for Injection USP, 0.9%) will be used to prepare this dose.
- Placebo for qHPV should not be diluted or mixed with other vaccines.
- Using aseptic technique, the pharmacist must withdraw 0.5-mL of Sodium Chloride for Injection, 0.9% using a sterile needle and syringe.

- Once drawn into the syringe, the placebo should be kept cold (2° to 8° C) until it is ready to be administered to the participant. Use promptly.

- The pharmacist will place an overlay on the syringe. With the overlay in place the syringe should be labeled as *"Quadrivalent HPV Vaccine or Placebo"* to maintain blinding. The syringe should also be labeled with the date/time product was withdrawn from the refrigerated vial into the syringe and the participant's identifier.

- All used vaccines should be discarded appropriately.

5.**5** Pharmacy: Product Supply, Distribution, and Accountability

5.**5**.1 Study Product Acquisition/Distribution

Quadrivalent HPV (types 6, 11, 16, and 18) recombinant vaccines for all participants are supplied by Merck & Co., Inc. The placebo for quadrivalent HPV vaccine (Sodium Chloride for Injection USP, 0.9%) must be obtained by the site.

5.**5**.2 Study Product Accountability

The site pharmacist is required to maintain complete records of all study vaccine received from Merck and subsequently dispensed. Continuous inventory is not required for normal saline, but all other information must be completed (including the lot number for the vial/ampule/IV solution used).

5.6 Concomitant Medications

5.6.1 Required Medications

None.

5.6.2 Prohibited Medications

- Receipt of licensed or experimental HPV vaccines outside of the study.

5.6.3 Precautionary Medications

- Anticoagulants other than aspirin or NSAIDS.
- Systemic antineoplastic or immunomodulatory treatment, systemic corticosteroids, other than inhaled corticosteroids, investigational vaccines, interleukins, interferons, growth factors, or intravenous immunoglobulin (IVIG).

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Events for all participants

	Screening	Entry	Week 4 (-0 days to plus 30 days)	Week 26 (-14 days to +45 days days)	Week 52/Premature discontinuation (+/- 45 days)
Documentation of HIV	X				
Medical history/Clinical Assessments	X	X	X	X	X
Stored Cervical HPV DNA PCR		X		X	X
Cervical cytology		X[2]		X	X
Cervical Colposcopy and directed biopsies	X[1]			X	X
LEEP/LLETZ			X		
HPV vaccine or placebo		X	X	X	
Pregnancy test	X	X	X	X	X
Plasma HIV-1 RNA/ CD4+/CD8+	X[1]				
Immunology subset study (n=120)					
Cervical sponge		X	X	X	X
Recut histology slides from biopsy for immunochemistry		X	X	X	X
Stored PBMCs /CD4 count		X	X	X	X
Stored serum for HPV antibodies, immune markers		X	X	X	X
Anal swab		X		X	X

	Screening	Entry	Week 4 (-0 days to plus 30 days)	Week 26 (-14 days to +45 days days)	Week 52/Premature discontinuation (+/- 45 days)
Stored plasma for HIV-1 RNA		X	X	X	X

1 Only needed if results are not available from within 90 days of entry
2 Only needed if results are not available from within 180 days of entry

6.2 Timing of Evaluations

6.2.1 Screening Evaluations

Screening evaluations must occur before any study treatment or intervention.

Screening evaluations to determine eligibility must be completed within 90 days prior to entry, unless otherwise specified. A cervical colposcopy/biopsy, CD4+/plasma HIV-1 RNA done as part of routine care meets this requirement if done within the 90 days prior to entry, and cytology meets this criteria if done within 180 days of entry. If patient has clinical sexual transmitted disease then the patient may be deferred until clinically resolved and then screening may continue.

6.2.2 Entry Evaluations

Screening may occur on the same day as entry if all necessary evaluations are available. Entry evaluations must occur after randomization and be completed before initiating study vaccine.

Entry evaluations may occur the same day as randomization.

All entry specimens must be obtained before initiating study treatment.

The participant should receive the first vaccine/placebo injection on the day of randomization. The vaccine must be given within 7 days after randomization if the vaccine.

6.2.3 Post-Entry Evaluations

If clinically active sexually transmitted Disease (STD) then the STD should be treated and then the samples collected after the STD clinically resolved. This should occur with 30 calendar days of the study visit in which the main and the immunology subset study testing should have been day

<u>On-Study Evaluations</u>
Study visits must be scheduled on the weeks indicated in the Schedule of Evaluations (SOE)

<u>Week 52 Final Visit Evaluations</u>
These evaluations are done at the participant's final on-study visit.

6.2.4 Discontinuation Evaluations

<u>Evaluations for Registered/Randomized Participants Who Do Not Start Study Vaccine</u>
No further evaluations should be performed. These participants will be replaced.

Premature Treatment Discontinuation Evaluations
No extra visit is required for those who initiate but do not complete the vaccination series. For those discontinuing the vaccine series after the first vaccine, the week 4 should be performed except for vaccine administration. The week 26 visit should be performed and the patient continued to be followed on study.. For those discontinuing the vaccine after the second vaccine, the week 26 visit should be performed. Please note that the week 26 and week 52 visits should be conducted even if the vaccine series is not completed.

Premature Study Discontinuation Evaluations
Participants who prematurely discontinue prior to the Week 52 window (- 45 days) should have the Week 52 visit performed early provided it has been at least 12 weeks since the date of the Week 28 visit.

6.3 Instructions for Evaluations

All clinical and laboratory information required by this protocol is to be present in the source documents.

All stated evaluations are to be recorded on the CRF and keyed into the database unless otherwise specified. This includes events that meet the International Conference on Harmonisation (ICH) definitions for a serious adverse event (SAE). See section 11.2.

To grade diagnoses, signs and symptoms, and laboratory results, sites must refer to the DAIDS AE Grading Table, Version 1.0, December 2004 (Clarification, August 2009), available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/ Grading Table).

Cytology and histology results, other than invasive cancer, do not need to be recorded as events. These results will be entered on study-specific CRFs.

6.3.1 Documentation of HIV-1

Refer to section 4.1.1 regarding assay requirements for HIV-1 documentation.

6.3.2 Medical History

The medical history must document any AIDS-defining illness or condition as defined by the CDC.

Record the participant's nadir CD4+ count (absolute value and date). Document the nadir CD4+ count when possible with a copy of the nadir CD4+ count report. If this documentation is not available, then subject recall will suffice. For participants who do not know the exact nadir value and for whom there is no source documentation, then recall of the categorical nadir (e.g., < 50, < 100, or < 200 cells/mm^3) will suffice.

Any prior history of treatment for cervical dysplasia or condylar should be documented.

6.3.3 Clinical Assessments

Signs and Symptoms

At entry, record all Grade 3 or greater signs and symptoms that occurred within 14 days before entry in source documents only. After entry, record Grade 3 or greater signs and symptoms regardless of their relationship to study treatment or study procedures in the source documents, and all signs and symptoms that led to a change in study treatment, regardless of grade. Please see section 6.3.5 for signs and symptoms to be recorded on the CRF.

Diagnoses

After entry, sites will only record the following:

- Any diagnosis of a cellulitis, abscess, or other infection at the vaccination site.
- Record all admissions for LEEP/LLETZ complications
- Record all cancer of the anogenital area

Concomitant Medications

Record the following:

- For the immunology subset, any ART interruption lasting ≥ 14 days during the study (from enrollment visit onward).
- Any precautionary or prohibited medications (see section 5.4) including anticoagulants other than aspirin or NSAIDS.
- Any topical or surgical treatments for CIN, VIN, VaIN, or genital condyloma
- Any HPV vaccination given outside of this study (this is prohibited but if given, needs to be recorded as a concomitant medication).

6.3.5 Laboratory Evaluations and Study Procedures

- After entry, record all Grade 3 or greater laboratory values if drawn to evaluate potential AEs related to study vaccination.
- Record all signs, symptoms or toxicities that led to a change in study vaccination, regardless of grade.
- Record all grade 3 and above AEs if deemed possibly, probably or definitely related to study vaccine or study procedures.

  Study visits collecting cervical specimens (i.e. swabs, lavage, fluid, biopsies or LEEP/LLETZ) should be deferred when the participant is menstruating. These visits should also be deferred if the participant exhibits visual evidence of bacterial sexually transmitted diseases other than bacterial vaginosis for 7 days after appropriate treatment is initiated and the study visit should be completed within 30 days of the initial study visit.

Cervical Cytology

Collected per the schedule of events using a cytobroom and placed on a glass slide (conventional cytology). These results will be interpreted by cytologist at the University of the Witwatersrand/NHLS (National Health Laboratory Services), a laboratory accredited with the South African National Accreditation System (SANAS). Previous quality assurance data on the previous Pap smears with this team of cytologists has shown a high level of concordance with cytology readings from University of North Carolina (85%).

For quality assurance, a second pathologist from South Africa will reread and the original Pap smear slide for diagnosis will not leave South Africa:

- 10% of screening/baseline cytology smears
- 100% of HSIL cytology specimens at weeks 26 and 52
- 20% of normal cytology specimens at weeks 26 and 52

Any discordant results will be adjudicated in conference.

Cervical HPV DNA PCR

Collected per the schedule of events. Cervical swabs should not be obtained during the 14 days after any cervical biopsy.

Cervical colposcopy and directed biopsies

Colposcopy will follow the cervical cancer research standard operational procedures. The patient is placed on the table with her feet in stirrups to position the pelvis for examination and the speculum is then inserted into the vagina to open vaginal walls and visualise the cervix. A light and low powered microscope (colposcope) is used to illuminate the cervix. The cervix is gently swabbed with 5% acetic acid to remove mucus and highlight abnormal areas like abnormal blood vessels, acetowhite epithelium and warts. Digital photographs and samples using biopsy forceps are then taken from all areas of suspicion and sent for histology in a container with formalin. Lugol's Iodine may be used if further elucidation of the suspicious area is needed. If no lesions are visualized at the week 26 visits, then no biopsies are taken. If no lesions are visualized at the week 52 visit, then biopsies at 6 and 12 o'clock will be taken. At least two biopsies are taken for each participant at week 52. If bleeding occurs, hemostasis is done with a dry swab held by forceps. Rarely, a hemostatic solution called Monsel's solution will be applied to reduce any minor bleeding. Inadequate colposcopy results will be repeated.

If participants require anticoagulation other than aspirin or NSAIDS after study entry, they should not undergo cervical colposcopy and biopsies.

Histology will be performed by the Department of Anatomical Pathology at the University of the Witwatersrand NO HISTOLOGY BLOCKS WILL LEAVE

SOUTH AFRICA. For quality control purposes, and second pathologist will reread:

- 25% of HSIL cervical biopsies used for eligibility,
- 25% of LEEP/LLETZ specimens, and
- 100% of cervical HSIL on biopsy at weeks 26 and 52
- 10% of non-HSIL biopsies at weeks 26 and 52

Cervical Fluid Collection (immunology subset only)
This should be done prior to the cytobroom for cytology and cervical swab for stored HPV DNA PCR. First, a speculum is inserted into the vagina exposing the cervix. Using a hemostat, one ophthalmic sponge is gently inserted into the cervical os being careful not to touch the vaginal walls and left in place for one minute. Then the sponge is removed, placed in a cryovial and frozen at -80 deg C.

LEEP/LLETZ
During the LLETZ procedure, the cervix is stained with 5% acetic acid and inspected under magnification. Abnormal areas are identified and subsequently stained with Lugol's Iodine. Local anaesthetic (Xylocaine 2% with adrenalin) is administered with a dental syringe and a 27G needle at the usual 3, 6, 9 and 12 o'clock positions around the cervix. A selection of a loop electrode is made depending on the size of the lesion that needs to be removed.

LLETZ is performed by a trained physician (either OB/GYN or Medical Officer trained in LLETZ) using a Finesse II ElectroSurgical Unit (ESU) with smoke evacuation system. The Finesse II ESU incorporates Controlled Output Circuitry to produce the best histological tissue specimen for the pathologist. Controlled Output Circuitry is an "intelligent cut" module that maintains the power output within the ideal cutting range to produce a specimen with minimal thermal damage at the margins. The generator continuously adjusts itself to meet the minimum needs for optimal cutting. This eliminates any need to adjust the output setting during the procedure, or when changing loop sizes. The smoke evacuation port is connected to the non-conductive speculum and the electrode is introduced into the vagina without activation. Only once in front of the cervix, is the loop electrode activated into cutting mode before touching the tissue, thereby activating the smoke exhaust fan before smoke can develop.

Under direct colposcopic vision, the loop electrode is introduced into the cervical tissue with gentle pressure, the operator starts cutting approximately 2 mm outside the identified abnormal epithelium. With a steady sweep the loop electrode is moved from one side of the cervix to the other, removing the previously identified lesion. If necessary, a second or even third sweep though the cervical tissue is made to remove any residual abnormal tissue. The specimen is immediately preserved in Formalin, properly labelled and prepared for transport to histopathology. hemostasis is achieved with electrocoagulation of

the crater bed using the same Finesse II ESU in coagulation mode with a ball electrode.

Pregnancy Test
A serum or urine pregnancy test is required for all women of reproductive potential (defined in 4.1.8) within 48 hours of entry, before each vaccination, before LEEP/LLETZ, and at any time pregnancy is suspected. Record the results of all pregnancy tests.

NOTE: Refer to section 7.3 for clinical management of women who become pregnant on study.

Plasma HIV-1 RNA
At entry, HIV-1 RNA determinations will be obtained. Samples for plasma HIV-1 RNA will be stored for the participants in the immunology subset at week 4, 26 and 52.

CD4+/CD8+
At screening, absolute CD4+/CD8+ count and percentages will be obtained. Samples for CD4+/CD8+ will be stored for the participants in the immunology subset at week 26 and 52.

Stored Serum (immunology subset only)
Serum for measures of HPV infection will be collected and stored per the SOE. These samples will be tested for soluble markers of inflamation and anti-HPV antibody concentrations once funding is identified. This will be done on the first 120 women who consent and qualify for the sub study

Stored PBMCs (immunology subset only)
PBMCs for future HPV and HIV immunology testing will be stored per the SOE. This will be done on the first 120 women who consent and qualify for the sub study.

Stored Anal HPV swabs (immunology subset only)
Anal HPV swabs will be stored per the SOE. This will be done on the first 120 women who consent and qualify for the sub study

6.3.8 Vaccine/Placebo Injection

Every effort should be made to administer vaccines within the protocol-defined visit window. Participants who do not receive the week 4 or week 26 vaccine will remain on study and continue the evaluations as listed in the SOE (see section 6.2.4).

The timing of the HPV vaccines is based on the Advisory Committee on Immunization Practices recommendations for HPV vaccination. If the week 4

and/or week 26 vaccine is not given within the specified windows, then the following guidelines must be followed:

- The week 4 vaccine must be given at least 28 days after the first vaccine. If the week 4 is not within the protocol-specified window, then the vaccine can be given as late as 24 weeks post randomization.
- The week 26 vaccine should be administered even if the week 4 vaccine is not administered because of missed visits.
- The week 26 vaccine must be given at least 12 weeks after the week 4 vaccine, and at least 24 weeks after the week 0 vaccine. The week 26 vaccine can be given as late 36 weeks post randomization.
- The week 52 visit should occur at least 12 weeks after the week 26 visit.

Vaccination should be deferred at the discretion of the site investigator if the participant is febrile or acutely ill.

Participants will remain at the clinic for a 15-minute post-injection observation period following each vaccination to observe for syncope or any immediate adverse reactions.

7.0 CLINICAL MANAGEMENT ISSUES

7.1 Dose Modification

No modification of the study vaccine dose is allowed.

7.2 Toxicity Management

7.2.1 Management of Injection Site and Allergic Reactions

Local and systemic reactions will be graded according to DAIDS AE Grading Table, Version 1.0, December 2004 (Clarification, August 2009).

<u>Injection Site Reactions</u>

- Swelling, induration, or redness at the injection site
- Pain or tenderness, or other reactions at the injection site

<u>Systemic reactions occurring within 48 hours after vaccination</u>

- Fever or chills
- Malaise or fatigue
- Headache or pain, e.g., myalgia or arthralgia
- Nausea or vomiting
- Allergic reactions, e.g., rash, hives, dyspnea, pruritis
- Syncope

For injection reactions judged to be severe, the protocol chairs should be notified within 24 hours and further vaccines should not be given to that participant prior to consultation with protocol co-chairs.

For injection reactions judged to be life-threatening, the protocol chairs should be notified within 24 hours and no additional vaccinations shall be given to that participant.

The protocol chairs should be contacted within 24 hours for any Grade 3 or 4 reactions (e.g., injection site reactions, elevated temperatures following vaccination that occur at any point during study participation) thought definitely, possibly, or probably related to vaccination. Further vaccines should not be given to that participant prior to consultation with the protocol co-chairs.

7.2.2 Other Adverse Events

AEs related to LEEP/LLETZ or cervical biopsies should be graded using the DAIDS "Female Genital Grading Table for Use in Microbicide Studies," which can be found on the DAIDS RSC web site: http://rsc.tech-res.com/safetyandpharmacovigilance/.

For toxicities not specifically addressed above, the following guidelines should be used for the management of AEs that are felt to be at least possibly related to vaccines:

- Grade 1 or 2 Toxicity/AE

  Participants who develop a Grade 1 or 2 AE may continue study vaccine. For instructions regarding participants experiencing Grade 1 or 2 AEs who choose to permanently discontinue study treatment or study participation, see section 6.2.4.

- Grade 3 or 4 Toxicity/AE

  Participants who develop a Grade 3 or 4 AE that is at least possibly related to vaccine should be reevaluated for that toxicity until the AE returns to Grade ≤2. The study vaccine may be given at the discretion of the site investigator. If the same Grade 3 or 4 AE recurs and is considered by the investigator to be possibly, probably, or definitely related to the study vaccine, the study vaccine must be permanently discontinued. If, in the investigator's opinion, the AE has NOT been caused by the study vaccine or if the event is an asymptomatic laboratory abnormality, study treatment may continue.

  For instructions regarding participants experiencing Grade 3 or 4 AEs that are at least possibly related to vaccine and that result in premature

discontinuation of study vaccine or study participation, see sections 6.2.4. These participants should be followed weekly until resolution of the AE.

7.3 Pregnancy and breastfeeding

Notify the protocol chairs of all on-study pregnancies or occurrences of breastfeeding on study.

Women who become pregnant on study will discontinue study treatment. Study visits should be suspended until the pregnancy has been completed. In the case of an early termination of pregnancy, the participant may continue with evaluations and the vaccination schedule per the SOE. Intrapartum complications and/or pregnancy outcomes will be recorded.

If a woman reaches week 52 or chooses to discontinue the study before the end of pregnancy, site staff should request permission to contact her regarding pregnancy outcomes. Information obtained will be submitted on the CRF at the end of the pregnancy.

Women who become pregnant on study through 6 weeks after the last study vaccine may request unblinding (see section 11). These cases of exposure to HPV vaccine will be reported Merck in accordance with Section 11.

8.0 CRITERIA FOR DISCONTINUATION

8.1 Premature Treatment Discontinuation

- Request by participant to terminate treatment.
- Treatment-related AEs as discussed in section 7.2.
- Pregnancy or breastfeeding.
- Clinical reasons believed life-threatening by the site investigator, even if not addressed in section 7.2.

8.2 Premature Study Discontinuation

- Request by the participant to withdraw.
- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the participant.
- Failure to start study vaccination within 7 days after randomization.
- At the discretion of the EC, industry supporter, investigator, or other government agencies as part of their duties to ensure that research participants are protected.

9.0 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

This is a randomized, double-blinded, placebo-controlled trial to assess the effect of pre-treatment HPV vaccination on the occurrence of cervical HSIL post-LEEP/LLETZ.

Quadrivalent HPV vaccine (6, 11, 16, and 18) will be administered in three doses with the second and third doses administered at weeks 4 and 26. The primary endpoint is defined as having cervical HSIL on cytology or biopsy at week 26 or 52.

The primary analysis is modified intention-to-treat. We will exclude randomized women who do not receive the first dose of study vaccine. These women will not be followed and will be replaced. Women to attend both the week 26 and week 52 visit will be censored from the primary analysis.

The targeted accrual is 180 women. We anticipate that accrual will be completed within one year.

9.2 Outcome Measures

9.2.1 Primary Outcome Measure

Cervical HSIL (HSIL on cervical cytology or HSIL on cervical biopsy) at week 26 or week 52.

9.2.2 Secondary Outcome Measures

9.2.2.1 Cervical cytology abnormalities at pre-entry, week 26, and week 52; and cervical biopsy abnormalities at week 26 and week 52.

9.2.2.2 Cervical swabs for high-risk HPV testing at entry, week 26 and week 52.

9.2.2.3 Cervical HSIL at week 26 and 52 in women stratified by whether cervical HSIL is found on LEEP/LLETZ biopsy, and stratified by whether LEEP/LLETZ margins gave HSIL detected.

9.2.2.4 Cervical sponge samples, recut slides from the cervical biopsy (samples from paraffin blocks collected at screening and post-entry, paraffin blocks from LEEP/LLETZ specimens at week 4, 26,52), PBMC, and whole blood lysate samples.

9.3 Randomization and Stratification

Participants will be randomized 1:1 to the HPV vaccination or placebo group using random permuted blocks. Randomization will be stratified based on participation in the Immunology Sub study.

9.4 Sample Size and Accrual

The primary analysis will be based on comparing proportions with cervical HSIL detected. We will perform the analysis at a two-sided 0.05 significance level and target an 80% power to detect a 50% reduction in hazard with vaccination assuming 40% occurrence of cervical HSIL in the placebo group and 20% occurrence in the placebo group. Alternate scenarios are shown below.

Probability of endpoint in control group	Probability of endpoint in vaccine group	Power to detect difference
50%	25%	92%
50%	30%	74%
40%	**20%**	**80%**
40%	25%	53%
30%	15%	63%

Reimers et al[33] reviewed 75 HIV-infected women who underwent LEEP/LLETZ for treatment of cervical HSIL. They found that 60% developed persistent or recurrent cervical HSIL. CD4 count was strongly associated with recurrent/persistent disease. The women in the study by Reimers et al had lower CD4 counts and less use of ART than women we expect to enroll in the proposed study. We expect a lower treatment failure rate for our proposed study. Firnhaber and colleagues are conducting a clinical trial comparing LEEP/LLETZ and cryotherapy treatment for cervical HSIL in HIV-infected women. When combining participants randomized to LEEP/LLETZ and women with HSIL who were ineligible for randomization for having lesions that are no amendable to cryotherapy (i.e. extensive or endocervical lesions), we estimate that 40% will have disease recurrence as defined by the primary endpoint.

9.5 Monitoring

This study will be reviewed every 2 months by the protocol co-chairs. They will review accrual, retention, pregnancies, study conduct (% of participants completing LEEP/LLETZ and vaccinations, % of expected visits completed and % of expected results received), and safety (Grade 3 or greater events at least possibly related to study vaccination, LEEP/LLETZ, or cervical biopsies).

We will constitute a Data and Safety Monitoring Board to review study conduct and safety. They will conduct a review approximately 6-9 months after study accrual has begun, and approximately one year later. No interim efficacy analysis is planned. The study accrual should be completed within one year. It will take one year until week 26 visits have been completed on 50% of participants. At this point, accrual will be

complete or nearly complete. Two or more Grade 3 or 4 adverse events or a single death that are at least possibly related to study vaccination will prompt an interim review by this advisory committee. Accrual will be halted until the Advisory Committee and the protocol chairs have reviewed the clinical trial data and agreed that the study should continue.

9.6 Analyses

9.6.1 Primary Analysis

The primary endpoint is the occurrence of cervical HSIL at week 26 or week 52. The timing of the endpoint at week 26 or 52 is not felt to be clinically relevant. We will not use time-to-event analyses. Only women who have completed a week 26 or 52 visit will be included in the analysis. We will compare the proportion of these women with cervical HSIL detected between arms. We will consider the number of missing week 26 or 52 visits in both arms. If there is an appreciable difference between arms, we will consider sensitivity analyses that adjust for missing visits.

Women who do not receive the first vaccine will be excluded from study follow-up and the primary analysis.

9.6.2 Secondary Analysis

9.6.2.1 Cervical cytology abnormalities will be summarized and reported according to randomized group at screening, week 26 and week 52.

Cervical biopsy results will be summarized and reported according to randomized group at week 26 and week 52.

LEEP/LLETZ biopsy results will be reported.

9.6.2.2 We will report on the availability on the availability of stored swabs for HPV testing at entry, week 4, week 26 and week 52.

9.6.2.3 We will perform the primary analysis in various subgroups. These subgroup analyses are not powered to show statistical significance. The focus will be on the direction of the point estimate and to better understand any observed efficacy. The subgroups include women with HSIL detected on LEEP/LLETZ specimen, and all other women. Please note that this subgroup relies on data observed at week 4. We do not expect that vaccination will affect the occurrence of HSIL on LEEP/LLETZ specimen based on published literature. We will look for appreciable differences in the prevalence of HSIL on LEEP/LLETZ specimens between groups. Any appreciable differences will limit the utility of this

analysis. A second subgroup analysis will be women with LEEP/LLETZ margins positive for HSIL, and all other women. This also involves a post-randomization variable and similar concerns exist for this subgroup analysis.

9.6.2.4 We will report the availability of Cervical sponge samples, cervical biopsy recut slides, paraffin blocks from LEEP/LLETZ specimens on week 4, PBMC, and whole blood lysate samples.

10.0 PHARMACOLOGY PLAN

Not applicable.

11.0 ADVERSE EVENT REPORTING REQUIREMENTS FOR THIS STUDY

11.1 Definitions

Adverse Event (AE) shall mean any untoward medical occurrence in a Study subject who is administered the Study Drug regardless of whether or not a causal relationship with the Study Drug exists. By way of example and without limitation, an AE can be any unfavorable and unintended sign (for example, an abnormal laboratory finding), symptom, or disease temporally associated with the use of the Study Drug,

Serious Adverse Event (**SAE**) shall mean any untoward medical occurrence in a Study subject who is administered the Study Drug that results in death, a life-threatening drug experience, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect, cancer, or is a new cancer if the cancer is the condition of the study, or overdose. Other important medical events that may jeopardize the patient or may require intervention to prevent one of the outcomes listed previously should also be considered "serious".

Suspected Unexpected Serious Adverse Reaction (**SUSAR**) shall mean any Serious Adverse Event, the nature, severity or frequency of which is not consistent with information in the most current investigator's brochure, or with respect to a marketed product the most current Summary of Product Characteristics (SPC) or Package Insert.

11.2 Reporting Requirements

11.2.1 SAE, SUSAR, pregnancy/breastfeeding, and cancer reporting**:** Protocol Chair shall forward to MSD's Global Safety ("MSD GS") group, any SAE and SUSAR information, including, but not limited to, all initial and follow-up information involving any participant. Notification shall be in the form of a completed CIOMS I/MedWatch within two (2) business days of learning of the SAE or SUSAR. All SAE and SUSAR information shall be transmitted in the English language and contain the

reporter's name and the participant identifier. SUSAR information will be reported unblinded.

Please note that all of SAE, SUSAR, and cancer diagnoses should also be reported to the EC and MCC within 24 business hours of site awareness. Weekends and public holidays are excluded.

All reports of study Drug exposure during pregnancy or breastfeeding, whether associated with an AE or not, must be reported to MSD GS in accordance with the timelines and contact information for an SAE. Study staff shall follow pregnancies to term to obtain the outcome of the pregnancy. The outcome of the pregnancy shall be forwarded to MSD GS.

All cancers should be reported as SAEs. Study drug overdoses will not be reported as SAEs unless there is an associated sign, symptom or diagnosis qualifying as an SAE. An inadvertent overdose will be recorded on the CRF. An overdose of vaccine/placebo is defined as administration of a 4th dose of vaccine/placebo during study participation.

11.2.2 Reporting Procedures to Government Agencies.

Adverse Reporting Department at Merck will determine the AEs reported to Merck that will be passed on to the US Agency, EMEA or other agencies by Merck. Local reporting to the ethics committee and South African health authorities is the responsibility of the protocol chairs.

11.3 Grading Severity of Events

The DAIDS AE Grading Table, Version 1.0, December 2004 (Clarification, August 2009), will be used. It is available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/.

AEs related to LEEP/LLETZ or cervical biopsies will be graded using the DAIDS "Female Genital Grading Table for Use in Microbicide Studies," which can be found on the DAIDS RSC web site: http://rsc.tech-res.com/safetyandpharmacovigilance/.

Cervical Cytology and biopsy results do not need to be recorded as AEs unless invasive cancer is shown.

11.4 Unblinding Procedure

Emergency unblinding is rarely allowed as the study product can be discontinued in a participant experiencing AEs without the need for unblinding. In the event that emergency disclosure of treatment assignment is thought to be required, the protocol

chairs should be contacted. The protocol chairs will discuss any unblinding request. If it is still thought to be necessary by one of the protocol chairs after this discussion, then the unblinding request will be granted. Should Merck have a request from an ex-US agency to unblind an SAE report, the request should come from Merck directly to study chairs and it will be handled as per the clinical trials agreement. Unblinding is allowed for women who become pregnant within 6 weeks after a vaccine dose. All SUSAR's will be unblinded.

12.0 HUMAN PARTICIPANTS

12.1 Ethics Committee Review and Informed Consent

This protocol and the informed consent document (Appendix I) and any subsequent modifications will be reviewed and approved by the EC responsible for oversight of the study. A signed consent form will be obtained from the participant or legal representative. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant or legal representative, and this fact will be documented in the participant's record.

12.2 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain participant confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will be released only as necessary for monitoring by EC, other government agencies, or the industry supporter or designee.

12.3 Study Discontinuation

The study may be discontinued at any time by the EC, the industry supporter, or other country-specific government agencies as part of their duties to ensure that research participants are protected.

13.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be determined by the study chairs. Submission of the primary manuscript is expected within 6 months of receiving the primary analysis.

14.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and

handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All dangerous goods materials, including diagnostic specimens and infectious substances, must be transported using packaging mandated by CFR 42 Part 72. Please refer to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

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