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Title: A Phase I Study of Quadrivalent Human Papilloma Virus (HPV) (Types 6, 11, 16, 18) Recombinant Vaccine in HIV-Infected and HIV-Negative Pre-Adolescents, Adolescents and Young Adults

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Investigational Agents:

Drug Name:	Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine (GARDASIL®)
IND Number:	13924 (cross ref Merck BB IND # 9030 and STN # 125126) NOTE: IND withdrawn April 2018.
Sponsor:	CCR
Manufacturer:	Merck & Co., INC

Commercial Agents:

None.

PRECIS

Background:

- Human papilloma virus (HPV) is one of the most common sexually transmitted diseases and a significant cause of cutaneous genital warts and anogenital cancer.
- Infection with high-risk, oncogenic HPV types, most commonly types 16 and 18, is associated with low and high-grade cervical cellular abnormalities that are precursors to invasive cervical cancer, as well as vulvar and anal cancer, while HPV types 6 and 11 are associated with genital warts.
- Persistence of HPV infection is more common in individuals with or at risk for chronic immunosuppression and HIV-infected individuals have a higher prevalence of HPV infection and HPV-associated anogential disease compared to age-matched HIV-negative controls.

Study Objectives:

- To assess the safety and immunogenicity of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in HIV-infected preadolescents, adolescents and young adults 12-26 years of age.
- To determine whether there are differences in HPV vaccine immunogenicity between HIV-infected and HIV negative age-matched controls.
- To determine whether there are differences in HPV vaccine immunogenicity between HIV-infected patients receiving highly active antiretroviral therapy (HAART) and those not receiving HAART with similar CD4 and viral load parameters at entry.
- To determine whether HPV vaccination alters HIV-1 RNA levels.
- To investigate the impact of CD4 count and HIV-1 RNA levels on HPV vaccine immunogenicity.
- To characterize HPV DNA positivity in the study cohort populations through oral/buccal and anogenital sampling at baseline.
- To characterize HPV and HIV knowledge and risk and sexual behaviors in the study cohort populations.

Eligibility:

Individual Cohorts

- Cohort 1: HIV-positive, CD4 cell count $\geq$ 350 cells/mm^3, HIV-1 RNA level by RT PCR $\leq$ 20,000 copies/ml, on stable HAART regimen for $\geq$ 6 months.
- Cohort 2: HIV-infected, CD4 cell count $\geq$ 500 cells/mm^3, HIV-1 RNA level by RT PCR $\leq$ 20,000 copies/ml, on no antiretroviral treatment.
- Cohort 3: healthy, HIV-negative controls

All Cohorts

- Females and males age 12 to 26 years
- Patients must have a hemoglobin $\geq$ 10.0 gm/dL, neutrophil count (ANC) $\geq$ 1500/mm^3, platelet count $\geq$ 75,000/mm^3 and PT or PTT $\leq$ 1.5X ULN (with the exception of patients with known clotting disorders or lupus anticoagulant); SGPT/SGOT $\leq$ 2/5X ULN, total bilirubin $\leq$ 1.5X ULN unless attributable to protease inhibitor therapy.

- Patients must test negative for hepatitis B virus and hepatitis C virus, unless the result is consistent with prior vaccination or prior infection with full recovery.
- No use of investigational agents within 4 weeks of study enrollment or use of immunosuppressive or immunomodulating agents within 8 weeks of study entry.

Study Design:

- This is a non-randomized, prospective, phase I study of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine.
- The study includes 3 cohorts of pre-adolescents, adolescents and young adults 12-26 years of age as outlined under Eligibility Criteria. Each cohort will enroll 35 patients.
- All study subjects will receive three doses of HPV vaccine at 0, 2 and 6 months administered IM.
- Study participants will be monitored at months 0, 1, 2, 3, 6, 7 and 12 (± 2 weeks for each visit) and every 6 months (± 30 days) thereafter for 48 months total.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

The ***primary*** objectives of this study are to:

- Assess the immunogenicity and safety of the quadrivalent human papillomavirus (types 6, 11, 16, 18) 3

The ***secondary*** objectives of this study are to:

- Determine whether there are differences in HPV vaccine immunogenicity between HIV-infected and HIV uninfected age-matched controls.
- Determine whether there are differences in HPV vaccine immunogenicity between HIV-infected patients receiving highly active antiretroviral therapy (HAART) and those not receiving HAART with similar CD4 and viral load parameters at entry.
- Determine whether HPV vaccination alters HIV-1 RNA levels.
- Investigate the impact of CD4 count and HIV-1 RNA levels on HPV vaccine immunogenicity.
- Characterize HPV DNA positivity by oral/buccal and anogenital swabs at baseline in the study cohort populations.
- Characterize HPV and HIV knowledge and risk and sexual behaviors in the study cohort populations.

1.2 BACKGROUND AND RATIONALE

1.2.1 Human Papillomavirus (HPV) Infection

Papilloma viruses are members of the Papovaviridae family of DNA viruses, all of which are considered to be potentially carcinogenic. Human papillomavirus (HPV) infection is the most common sexually transmitted infection in the world and is well established as a significant cause of anogenital cancer and cutaneous disease. In the United States, approximately 20 million people are infected with HPV, 1.4 million individuals currently have genital warts and 5.5 million new cases of HPV infection occur every year.[1] Overall, it is estimated that 75% of sexually active individuals have been exposed to HPV during their lifetime.[2] On the basis of variations in genotype homology, more than 100 HPV types have been identified (genotypes are defined by less than 90% homology of HPV E6, E7 and L1). However, only approximately one third of them infect the epithelial lining of the anogenital tract and other mucosal areas of the body; of these, 15 types (16, 18, 31, 33, 35, 39, 45, 51,52, 56, 58, 59, 68, 73, 82) are considered to be high-risk, oncogenic types. Types 26, 53, and 66 have recently been classified as probable high-risk types,[3] and additional high- and low- risk HPVs continue to be identified. High-risk HPV types are associated with high-grade epithelial lesions and cancer. Low-risk types are associated with genital warts, low-grade epithelial lesions, and recurrent respiratory papillomatosis. Individuals may be infected with more than one HPV type at any given time and co-infection with multiple HPV types is a common finding of many epidemiologic studies. Persistent infection with high risk, oncogenic HPVs is now unequivocally associated with the development of cervical cancer (particularly with HPVs 16 and 18) and is likely to be responsible for a substantial proportion of other anogenital (vulva, vagina, penis and anus) and aero-digestive tract cancers. Low risk HPV types, predominantly HPV 6 and 11, are also responsible for considerable healthcare utilization and morbidity as the cause of genital warts, whose incidence appears to be rising.[4]

HPV infections are often sub-clinical making them difficult to detect and prevent. Although consistent condom use appears to reduce the risk of cervical and vulvovaginal HPV infection in sexually active young women,[5] they are not completely effective because the virus can still exist on areas of the genital region that are not typically covered by male condoms. Risk factors for HPV transmission include young age, having multiple sex partners, the presence of other sexually transmitted (e.g. Chlamydia, herpes simplex virus) or genital tract (e.g. bacterial vaginosis) infections, combining sex with alcohol, smoking, and having sex with men who have had many sexual partners.[6,7,8] Other mediating factors including oral contraceptive use, chronic inflammation, immunosuppressive conditions including HIV infection, parity, dietary factors and human leukocyte antigen polymorphisms.[9] Many epidemiological studies have documented that the prevalence of HPV infection peaks among sexually active women less than 25 years of age[10], with a subsequent age-related decline in prevalence observed thereafter. However, some studies have also documented a second peak in HPV prevalence beginning around age 45-50 in peri- or post-menopausal women.[11,12] Although the reason for this second, menopausal peak is unclear, non-mutually exclusive hypotheses include reactivation of latent infections acquired early in life due to loss of HPV type-specific immunity; acquisition of new infections due to sexual contacts with new partners later in life or diverse HPV exposure of successive birth cohorts.

While HPV infections are common, most infections appear to clear spontaneously: cohort studies have consistently documented that only a small proportion of women positive for a given HPV type are found to have the same HPV type in subsequent specimens.[9] It is unclear whether host immune responses are able to clear infections completely or if the virus remains latent in basal cells or some other unidentified reservoir at undetectable levels. What is clearly established is that the risk of subsequent cervical intraepithelial neoplasia (CIN) is proportional to the number of specimens testing positive for HPV[13,14], suggesting that carcinogenic development results from prolonged exposure to sustained viral replication. Despite the definitive evidence that HPV persistence is a key intermediate in cervical carcinogenesis, there is no consensus regarding the definition of what constitutes a persistent versus a transient HPV infection. While many epidemiologic studies have sought to examine the natural history of HPV infection, there is no clear duration threshold that can be used to distinguish an episode of HPV infection that is transient from one that is persistent. Importantly, it is virtually impossible, especially for common HPV types such as HPV 16, to distinguish an instance of persistent infection from one that simply represents the *loss and subsequent reacquisition* of the same HPV type from the same or different sexual partner. Epidemiologic studies which have sought to determine the duration and clearance rates of individual episodes of HPV infection vary substantially: the average length of individual episodes ranges from 4 to 20 months, while clearance rates are between 10 and 60%.[9] However most studies indicate that less than half of women who develop an HPV infection will continue to be positive at one year.

HPV is a non-lipid enclosed virus with a circular, double stranded DNA genome. Its major capsid proteins are L1, the major target of prophylactic HPV vaccines, and L2. Microabrasions in the skin or mucosa allow HPV to establish infection in the basal cell layer of stratified squamous epithelium. Once infected, cells are induced by HPV to proliferate, divide and expand laterally. Viral gene expression and multiplication occur exclusively in the nuclei of the infected cells and are tightly linked to the differentiation state of the cells. In basal and parabasal cells, viral DNA replicates at a low level as an episome and only early genes are transcribed. As infected cells mature to the surface, extensive viral replication and DNA transcription of all viral genes as well as capsid and viral particle formation occurs in the most superficial layers of epithelium.[15] Upon reaching the upper epidermis or mucosa, viral particles are released where they can in turn infect adjacent tissue. Neoplastic transformation is associated with breakage of the circular viral genome- most often within the E2 gene, and integration

into the host genome. This integration interrupts E2 function, which normally down-regulates the transcription of the E6 and E7 genes and promotes the release of p53 and pRB proteins involved in control of cell cycle replication and DNA repair. During HPV-induced cell transformation, p53 and pRB are rendered inactive by E6 and E7 proteins[16,17], and the neoplastic transforming capacity of E6 and E7 is greatest for high-risk oncogenic types, particularly HPV 16 and 18.

The clinical manifestations of HPV infection include genital warts (condyloma accuminata), cervical, vaginal, vulvar and anogenital intraepithelial neoplasia, anogenital squamous cell cancers, and recurrent laryngeal papillomatosis, a rare but serious condition that affects infants and young children born to HPV-infected mothers. The most common sites of HPV infection are those associated with microabrasions as a result of micro- or macrotrauma during sexual activity, such as the perineum, posterior fourchette, and the perihymeneal area in women and the uretheral glands and penile shaft in men. Other areas at major risk for viral entry are epithelia that are naturally thin and immature, such as the transformation zone of the cervix or the anal verge. The single layer columnar epithelium associated with cervical ectopy that is the hallmark of pubertal maturation in adolescent women, makes this population uniquely vulnerable to HPV acquisition, persistence and malignant transformation. The incubation period for genital warts is highly variable, from as little as 3 weeks to 6 to 8 months or more following exposure. Genital warts can have a variety of appearances and are usually not associated with symptoms unless they are extensive or extremely exophytic. They are often subclinical when located on the cervix and are able to be visualized only with colposcopy after the topical application of 5% acetic acid.

Cervical HPV infection is typically subclinical and is only able to be detected through cervical cytology (pap smear), colposcopy, biopsy and HPV DNA testing. Cervical abnormalities are usually caused by high-risk HPV types, although these abnormalities often regress spontaneously and women infected with oncogenic types often have normal pap test results. The most common cervical HPV lesions are cervical dysplasia, also known as cervical intraepithelial neoplasia or CIN. CIN is classified into low-grade and high-grade categories: low-grade, CIN1, compromises mild dysplasia and cervical condyloma that cannot be reliably distinguished on cytology, colposcopy, or histology. Histologically, cells with perinuclear halos and nuclear atypia (termed koilocytes) are seen in the upper layers of the epithelium and are a hallmark cytopathic manifestation of HPV infection. High-grade dysplasia, CIN2 and 3, compromises moderate and severe dysplasia and are characterized by cells with nuclear atypia, high nuclear to cytoplasmic ratios and nuclear crowding extending to the upper layers of the epithelium. Colposcopy is warranted for pap smears documenting low-grade squamous intraepithelial lesions, atypical squamous cells suggestive of a high-grade lesion, high-grade squamous intraepithelial lesions, cancer, persistent atypical squamous cells of undetermined significance, or ASCUS cytology with a positive reflex HPV test. While regression is the norm for CIN1 lesions and only conservative clinical follow-up is warranted, CIN2/3 lesions are at high risk for progression to cervical cancer and must be treated.

1.2.2 HPV Infection in Children

HPV is transmitted by skin-to-skin contact, most commonly through sexual activity in both men and women. In adults, insertive sexual contact i.e. sexual transmission appears to be the primary route of transmission. While detection of genital HPV types in children is relatively uncommon, multiple studies have confirmed the detection of HPV in the pre-pubertal population although the frequency of detection reported is highly variable and the mode of HPV transmission is an ongoing source of debate.[18,19] Vertical or perinatal transmission from an HPV-infected maternal genital tract, horizontal transmission by auto- or hetero-inoculation from non-genital cutaneous warts and transmission by sexual abuse have

all been documented.[20] Although well-designed, prospective studies involving large numbers of infants detected HPV in only1.5%-3.7% of genital samples[21-23] suggesting that vertical HPV transmission is rare[24], several other studies have reported infection rates ranging from 4-72% among infants born to mothers testing positive for HPV DNA during pregnancy.[25-29] Detection of both high and low risk HPVs has been documented using PCR-based methodology. More recent studies examining HPV positivity in large cohorts of infants as well as their mothers and fathers [30,31], reported HPV DNA detection in 4%-15% of genital and 10-21% of oral samples at birth with overall acquired infection and persistence rates that were higher than have previously been reported.[31] Longitudinal follow-up suggested a peak in oral and genital HPV detection at 6 months followed by a decline by 24 months.[30] Despite the discrepancies in the frequency of HPV detection reported among infants, detection was universally more common in infants born to mothers positive for HPV DNA and in one study was associated with other obstetrical factors such as prolonged rupture of membranes $\geq$ 2 hours.[29] Seroepidemiologic investigations of HPV infection in a representative sample of U.S. children 6-11 years of age documented an overall seroprevalence rate of 2.4%, with higher rates in children > 7yrs and boys.[32] Despite variability in the frequency of HPV detection in children, the published literature clearly supports the contention that vertical transmission does occur and it is presumed to be the primary mode of transmission in children with recurrent respiratory papillomatosis (RRP)[33], an HPV-related condition that peaks in children aged 2-3 years.[34] Although rare, RRP can be a source of significant childhood morbidity due to repetitive surgeries for papilloma removal resulting in chronic inflammation, permanent vocal cord damage and even death associated with airway obstruction by papillomas or spread to the lung parenchyma.[35] Similar to anogenital warts, RRP is caused by low-risk HPV types 6 and 11.

1.2.3 Adolescent Sexual Behavior and HPV Infection

Adolescence is associated with an upsurge in sexual activity, pregnancy and sexually transmitted infections (STIs). According to CDC data, approximately 1 million teens become pregnant and 3 million teens are infected with STIs annually.[36] In addition, adolescents 10-19 years of age and young adults 20-24 years of age account for two thirds of all reported STIs. Many factors are responsible for the high rate of STIs in adolescents. They include personal behavioral factors such as age of sexual debut, multiple, concurrent or older partners, frequent change of partners, casual or anonymous partners, partners from groups with a high prevalence of STIs, sex associated with recreational drug use (especially alcohol) or substance abuse, sex in exchange for drugs or money, an inability to notify partners when appropriate that they require STD treatment, and inconsistent condom use.[37] Historically, the efficacy of latex condoms for reducing the risk of contracting sexually acquired HPV was not well established. A meta-analysis by Manhart and Koutsky found inconsistent evidence that condom use reduced the risk of becoming HPV DNA positive, although condom use overall was effective in reducing HPV-associated outcomes of genital warts, cervical dysplasia, and invasive cervical cancer.[38] A more recent prospective study of 82 female university students documented that among newly sexually active women, consistent condom use by their partners appeared to reduce the risk of both cervical and vulvovaginal HPV infection.[39]

Risk taking behavior and experimentation are the hallmarks of adolescent development and are often characterized by dissociation between risk behaviors and potential adverse outcomes as a result of cognitive and emotional immaturity i.e. the “It can’t happen to me” syndrome. Importantly, among adolescents, self-characterization of sexual or drug use behaviors is often different from self-identification e.g. youth do not self identify as homosexual or bisexual even though they have same sex partners or have sex with both men and women. In addition, both adolescent males and females engage

in a wide range of sexual behaviors including oral and anal sex. Social factors associated with increased STI acquisition risk include poor parent-adolescent communication, lack of parental supervision, insufficient sex education, lack of health insurance, absence of a user-friendly, confidential source of health care, dating violence and incarceration.[37] Among adolescent women there is also a biologic predisposition to increased STI acquisition associated with exposed columnar epithelium on the cervix, termed cervical ectopy or ectropion, a common condition in this age population. Other possible biologic factors include lack of protective antibodies as a consequence of previous infections; decreased levels of IgG during the follicular phase of the menstrual cycle compared with adults; and in younger adolescents, fewer protective hydrogen peroxide-producing lactobacilli in the genital tract.[40]

Although it is well understood that infection of the genital tract with HPV leads to a range of pathologic states, estimates of HPV prevalence are extrapolated from epidemiological studies measuring current infection and vary by the population studied, the case definition criteria for HPV infection (clinical vs. molecular) and the methodology used to detect HPV. Latent HPV infection diagnosed by DNA detection methods without any evidence of clinical disease is the most common manifestation of HPV in adolescents. In studies of adolescent females, the prevalence of latent HPV infection ranges from 13 to 64%, [37] with cumulative prevalence rates as high as 82%.[41] Of these infections, 40 to 75 percent are caused by high-risk viral types, with 16, 18, 52 and 59 identified most often in adolescents.[41,42] In addition, co-infection with multiple HPV types is seen commonly in adolescents[41] which in turn has recently been reported to be associated with an increased risk of precancerous cervical lesions in epidemiologic studies.[43] In general, adolescent males have been less well studied because of lack of standardized testing protocols for the male genital tract. While adverse health outcomes in males appear to be substantially less than those observed in women, males clearly function as a reservoir of HPV infection and re-infection for women, and HPV is well established as a cause of genital and anal-rectal cancers in men. Prevalence rates as high as 75 percent have been reported in asymptomatic, heterosexual adolescent males but vary according to the number of sites sampled (scrotum, urethra, anus or penis) and /or the inclusion of other specimens sampled (urine or semen).[44] However rates appear to be much higher in males who report having sex with other males (MSM) and are also related to sexual behaviors, particularly the number of lifetime sexual partners.[42]

1.2.4 HIV Infection and HPV Co-Morbidity

It has been recognized for many years that individuals with immunosuppression caused by HIV infection or organ transplantation are at increased risk of HPV-related anogenital cancers compared with age-matched healthy controls. Although the exact role of immunosuppression in conferring increased risk is not known, immunosuppression is typically associated with a higher frequency of incident and cumulative HPV infection- particularly with high-risk HPV types, persistence of HPV infection, cervical dysplasia and neoplasia, more rapid neoplastic progression, refractoriness to treatment and recurrence following treatment.[45] Specifically, HIV positive women are about five times as likely as HIV-seronegative women to have squamous intraepithelial lesions, vulvovaginal condyloma acuminata, or anal intraepithelial neoplasia.[46-52] In addition, HPV-associated disease is often multifocal in HIV positive women and may also involve the anus.[53] Despite the overwhelming epidemiologic and clinical associations, the mechanisms of HPV-HIV interaction and co-morbidity remain poorly understood and are still being defined. Excess HPV-related neoplasia likely reflects HIV-induced immunosuppression but may also result from direct interactions of HIV with HPV. In a multi-center, prospective cohort of HIV-infected women, risk factors for abnormal cytology included CD4 cell count and HIV-1 RNA level, in addition to other well-established risk factors such as detection of HPV, prior history of abnormal cytology and number of male sexual partners.[54] Although a similar higher incidence of HPV-

infection with high risk (but not low risk) types and squamous intraepithelial lesions (SIL) was documented in HIV-positive adolescent girls compared to HIV-negative girls, neither CD4 cell count nor viral load were associated with HPV infection or SIL.[55] However, only 7% (9 of 133) of the HIV-infected girls in this study had CD4 cell counts less than 200 cells/mm^3. In a subsequent natural history study, plasma HIV-1 RNA level and CD4 count in combination were confirmed to have a strong and statistically interactive association with incident detection of HPV, some of which was attributed to possible to HPV reactivation.[56] In response to the repeated observations of cervical cancers in HIV-infected women, moderate and severe cervical dysplasia were designated as indicative of early symptomatic HIV infection (Category B) and invasive cervical cancer as an AIDS-defining (Category C) condition by the Centers for Disease Control and Prevention (CDC) in 1993.[57]

Importantly, HPV infection is also a significant source of co-morbidity in HIV-positive men, particularly MSM. HIV-*negative* MSM have been shown to have a stable, high prevalence (57%) of anal HPV infection across all age groups, that is independently associated with receptive anal intercourse and having > 5 sex partners.[58] Accordingly, HPV infection in this population has been documented to be associated with a high prevalence of anal squamous intraepithelial lesions (ASILs), anal cancer precursors comprised of low-grade squamous intraepithelial lesions (LSILs) and high-grade squamous intraepithelial lesions (HSILs) identical to classification of cervical cancer precursors.[59] Detection of LSILs or HSILs were associated with infection with multiple HPV types. These observations continue to hold in men with HIV infection: incident HPV infection, infection with multiple HPV types and HPV-related anal cancer and ASILs all occur more commonly in HIV-positive men then in HIV-negative men.[60-64] Specifically, detection of multiple HPV types is associated with both prevalent anal intraepithelial neoplasia and progression to higher grade anal neoplasia over time.[60]

The introduction of highly active antiretroviral therapy (HAART) has resulted in a significant decline in HIV-related mortality and dramatic reductions in the incidence of opportunistic infections in both children and adults.[65-67] As a consequence, HAART-treated individuals are living longer and children with HIV disease acquired early in life are surviving into adolescence and young adulthood, making them potentially at increased risk of malignant complications because of prolonged immunosuppression. Data associating an increased risk of cervical disease with low CD4 cell counts and high HIV-1 RNA levels,[68-69], suggest that HAART should be beneficial to the natural history of cervical disease in HIV positive women. However, reported data on the natural history of cervical intraepithelial neoplasia (CIN) are mixed. Spontaneous regression of low- and high-grade lesions has been described in HIV positive women receiving no therapy as well as in those receiving HAART or non-HAART regimens.[70] However, in this cohort and others, HPV infection persists in up to 80% of the cases, suggesting that antiretroviral therapy does not prevent the development of HPV-associated lesions or eliminate HPV infection once established.[69,70] Despite the introduction of HAART, the incidence of anal cancer and AIN has continued to increase among HIV-positive MSM.[71] Although several studies have confirmed the increased risk of pre-invasive cervical lesions among HIV-infected women, there are currently no data available to shed light on the effect of HAART on the incidence of invasive cervical cancer in this population.[72] Hence, early and regular anogenital monitoring is warranted for both HIV-infected men and women to prevent and reduce the morbidity of HPV-related pre-neoplastic conditions and malignancy.

While the clinical imperative to conduct cervical and anal cancer screening in HIV-infected individuals is unequivocal, there has been conflicting evidence as to how well cervical cytology performs compared to coloposcopy.[73] In addition, there are currently no established, evidence-based, universal guidelines outlining HPV diagnostic screening or treatment recommendations for HIV-positive men or women.

Given the higher prevalence and incidence rates of CIN in HIV-positive women, the sensitivity of cervical cytology screening (Pap smears) can be improved by increasing the frequency of screening. Current CDC guidelines recommend that HIV-seropositive women receive two Pap smears at six month intervals within the first year of diagnosis followed by annual Pap smear tests if these are normal.[74] Others have advocated baseline colposcopy to examine the whole anogenital area due to the increased risk of vaginal, vulvar and anal intraepithelial neoplasia.[75] There are currently no tests approved to detect early evidence of HPV-associated cancers in men. Nonetheless, since anal cancer is more common in MSM and HIV-positive men, some experts recommend routine anal Pap tests for those populations and anoscopy when clinically indicated. It remains to be determined whether prospective anal cytologic screening in males will have the same clinical utility as cervical cytologic screening in women. At present the CDC does not recommend anal cancer screening.[76] The role of HPV DNA testing in addition to cytologic screening remains to be determined: there are some studies that suggest additional efficacy and cost effectiveness [77] while other studies have demonstrated that HPV testing only slightly improved the sensitivity and predictive value of baseline screening when compared with cytologic screening alone.[78]

1.2.5 Quadrivalent Human Papillomavirus (types 6, 11, 16, 18) Recombinant Vaccine

The quadrivalent HPV vaccine is a non-infectious recombinant vaccine prepared from the highly purified virus-like particles (VLPs) of the major capsid (L1) protein of HPV types 6, 11, 16 and 18.[79] The vaccine, produced by Merck & Co., and marketed under the name GARDASIL®, was approved and licensed by the FDA for clinical use on June 8, 2006. The L1 proteins are produced by separate fermentations in recombinant *Saccharomyces cerevisiae* and self-assembled into VLPs. In animal papillomavirus models, systemic immunization with L1 VLPs induces high titers of neutralizing antibodies that confer protection against high-dose experimental papillomavirus challenge.[80-82] As CIN 2/3 and AIS are the immediate and necessary precursors of squamous cell carcinoma and adenocarcinoma of the cervix respectively, and their detection and removal has been shown to prevent cancer, they thus serve as surrogate markers for the prevention of cervical cancer. Efficacy was assessed in 4 placebo-controlled, double-blinded, randomized Phase II and III clinical studies involving 2,942 and 17,599 subjects, respectively.[83] Efficacy was analyzed for each study individually and for all studies combined according to a prospective clinical plan. GARDASIL® was administered without prescreening for presence of HPV infection and the efficacy trials allowed enrollment of subjects regardless of baseline HPV polymerase chain reaction (PCR) status or serostatus. The primary analyses of efficacy were conducted in the per-protocol efficacy (PPE) population, consisting of individuals who received all 3 vaccinations within 1 year of enrollment, did not have major study deviations from the study protocol, and were naïve (PCR negative in cervicovaginal specimens and seronegative) to all 4 vaccine HPV types prior to dose 1 and through 1 month following dose 3 (Month 7). Efficacy was measured starting after the Month 7 visit. Overall, 73% of subjects were naïve to all 4 vaccine types at enrollment and 27% of subjects had evidence of prior exposure to or ongoing infection with at least 1 of the 4 vaccine HPV types.

GARDASIL® was efficacious against HPV disease caused by each of the 4 vaccine types in reducing the incidence of any grade CIN, including CIN 2/3, adenocarcinoma *in situ* (AIS), genital warts, vulvar intraepithelial neoplasia (VIN), and vaginal intraepithelial neoplasia (VaIN) in those subjects who were PCR negative and seronegative at baseline. Specifically, the efficacy of GARDASIL® against HPV 16/18-related disease was 100% for CIN 3, or AIS and 100% for VIN 2/3 or VaIN 2/3. The efficacy of

GARDASIL® against HPV 6-, 11-, 16-, and 18-related VIN or VaIN 1 was 100%. There was no clear evidence of protection from disease caused by HPV types for which subjects were PCR positive and/or seropositive at baseline. However, individuals who were already infected with 1 or more vaccine-related HPV types prior to vaccination were protected from clinical disease caused by the remaining HPV vaccine types.

The immunogenicity of GARDASIL® was assessed in 8915 women (GARDASIL® N = 4666; placebo N = 4249) 18 to 26 years of age and female adolescents 9 to 17 years of age (GARDASIL® N = 1471; placebo N = 583). Type-specific competitive immunoassays with type-specific standards were used to assess immunogenicity to each vaccine HPV type. These assays measured antibodies against neutralizing epitopes for each HPV type. The scales for these assays are unique to each HPV type; thus, comparisons across types and to other assays are not appropriate. The primary immunogenicity analyses were conducted in study subjects with the same characteristics as the PPE population. Overall, 99.8%, 99.8%, 99.8%, and 99.5% of girls and women who received GARDASIL® became anti-HPV6, anti-HPV 11, anti-HPV 16, and anti-HPV 18 seropositive respectively, by 1 month following vaccine dose 3 (Month 7) across all age groups tested. Geometric mean titers (GMT) to all vaccine HPV types peaked at Month 7, declined through Month 24 and then stabilized through Month 36 at levels above baseline. The duration of immunity following a complete schedule of immunization with GARDASIL®has not been established. Because there were few disease cases in study subjects naive to vaccine HPV types at baseline in the group that received GARDASIL®, it has not been possible to establish minimum antibody levels that protect against clinical disease caused by HPV 6, 11, 16 and/or 18. Importantly, co-administration of GARDASIL® with recombinant hepatitis B vaccine (same visit, separate injection sites) was evaluated in a randomized study of 1871 women 16 to 24 years of age and immune responses to both hepatitis B vaccine and GARDASIL® were documented to be non-inferior.

Although GARDASIL® vaccine has been studied in 9- to 15-year old boys, the data were considered insufficient at the time of FDA review to assess vaccine immunogenicity in males. GARDASIL® vaccine has not been given to individuals with HIV infection but this NCI study and other planned studies will contribute to the knowledge base regarding vaccine immunogenicity and safety in this population. The ACIP recommendations for the use of quadrivalent HPV vaccine[84] (**Appendix 1**) include:

- Routine vaccination with three doses of quadrivalent vaccine for females 11-12 years of age. The vaccination series can be started in females as young as 9 years of age.
- Catch-up vaccination is recommended for females 13-26 years of age who have not been vaccinated previously or who have not completed the full vaccine series. Ideally, vaccine should be administered before potential exposure to HPV through sexual contact.
- Each dose of quadrivalent HPV vaccine is 0.5ml, administered intramuscularly.
- Quadrivalent HPV vaccine is administered in a three dose schedule. The second and third doses should be administered 2 and 6 months after the first dose.
- Quadrivalent HPV vaccine can be administered at the same visit when other age appropriate vaccines are provided, such as Tdap, Td and MCV4.
- At present, cervical cancer screening recommendations have not changed for females who receive quadrivalent HPV vaccine.

The recommendations also advise that quadrivalent vaccine can be given to females who have an equivocal or abnormal pap test, a positive Hybrid Capture II® high-risk test, or genital warts. Vaccine

recipients should be advised that data from clinical trials do not indicate the vaccine will have any therapeutic effect on existing Pap test abnormalities, HPV infection or genital warts. Vaccination of these subjects would provide protection against infection with vaccine HPV types not already acquired. Importantly, the recommendations state that females who are immunocompromised either from disease or medication *can receive* quadrivalent vaccine, although the immune response to vaccination and vaccine effectiveness may be less than in females who are immunocompetent. The ACIP Provisional Recommendations make no comment regarding the administration of HPV vaccine to males.

The impact of quadrivalent vaccine on HIV-1 replication is unknown and will be examined as an endpoint in this study. Early reports of increased viral replication following influenza[85] and pneumococcoal vaccination[86] raised concerns that routine vaccination with common neo and recall antigens might lead to accelerated HIV disease progression. Multiple subsequent prospective studies documented that increases in plasma viral levels following immunization with a variety of antigens were either transient[87-90] or did not occur at all[91-93] despite HIV-infected individuals receiving no or suboptimal antiretroviral therapy (i.e. dual nucleoside analog RTI). With the advent of highly active antiretroviral treatment (HAART), additional large prospective studies have failed to document clinically significant acute or chronic increases in viral replication following vaccination.[94,95] Consequently, the ACIP and the American Academy of Pediatrics recommend that HIV-infected children and adolescents in the U.S. should receive routine pediatric immunizations according to the recommended schedule.[96] Since immunocompromised individuals can develop disease from vaccine strains of organisms found in live vaccines, HIV-infected children generally should not receive live virus or bacterial vaccines with the exception of measles-mumps-rubella (MMR) and varicella vaccine, and inactivated polio vaccine (IPV) should be given instead of live oral polio vaccine (OPV).[97]

1.2.6 Rationale for Study Cohort Population

This study will be conducted in both HIV-infected females and males 12-26 years of age. Since the risk of HPV acquisition is intensified during adolescence and young adulthood because of risk-taking and exploratory behaviors and because of the well-documented co-morbidities associated with sexually acquired HPV in individuals with HIV infection, both males and females are included in the study. It is anticipated that Cohort 1 will be comprised primarily (but not exclusively) of sexually naïve pre-adolescents and adolescents infected with HIV vertically or by transfusion early in life that have experienced successful immune reconstitution and viral load reduction on HAART therapy (i.e. CD4 count $\geq$ 350 cells/mm^3 and HIV-1 RNA levels $\leq$ 20,000 copies/ml). This population represents an ideal one to investigate vaccine immunogenicity given the high likelihood of HPV acquisition following the initiation of sexually activity.

It is anticipated that Cohort 2 will be comprised primarily (but not exclusively) of adolescents and young adults with sexually acquired HIV infection that have no current indication for HAART therapy according to current USPHS Treatment Guidelines (i.e. CD4 count $\geq$ 500 cells/mm^3 and HIV-1 RNA levels $\leq$ 20,000 copies/ml).[98] These individuals represent a unique population to assess HPV vaccine immunogenicity and safety that parallels populations in developing countries with little or no access to antiretroviral treatment where there is a high burden of prevalent HPV and HIV disease. Both Cohorts 1 and 2 will permit assessment of whether HPV vaccination is associated with alterations in HIV-1 RNA levels and if observed, whether alterations are mitigated in the presence of HAART. In addition, both HIV-infected cohorts will allow investigation of the impact of CD4 count and HIV-1 RNA levels on HPV vaccine immunogenicity.

Cohort 3 will be comprised of HIV-*negative* controls (total N=35) that will allow comparative analysis of vaccine immunogenicity, duration of responses, carriage of HPV DNA in oral/buccal and anogenital samples and risk behaviors compared to the HIV-infected cohorts (total N=70). Every effort will be made to enroll HIV-negative individuals that are representative for both age and gender compared to the HIV-positive population. HIV-negative individuals will be recruited through the NIH Clinical Center's Clinical Research Volunteer Program (CRVP) for healthy volunteers (301-496-4763 / 1-800-892-3276 or CRVP@mail.cc.nih.gov) and may also include HIV-negative siblings of HIV-infected patients. The CRVP has a well-established track record in the recruitment of both adult and pediatric/minor volunteers for NIH studies.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Cohort 1 Inclusion and Exclusion Eligibility Criteria

Inclusion Criteria

2.1.1.1 Age 12 to 26 years

2.1.1.2 Females and males

2.1.1.3 HIV-positive

2.1.1.4 CD4 cell count and HIV-1 RNA level parameters

2.1.1.4.1 CD4 cell count $\geq$ 350 cells/mm^3

2.1.1.4.2 HIV-1 RNA level by RT PCR $\leq$ 20,000 copies/ml

2.1.1.5 On stable HAART regimen for > 6 months with CD4 and viral load parameters as outlined in 2.1.1.4

2.1.1.6 Patients > 18 years willing to provide informed consent or parent/guardian willing to provide informed consent for minor children <18 years of age.

2.1.1.7 Informed assent for patients 12-17 years of age (Optional at the discretion of the Principal Investigator and Parent/Guardian based on maturity level of minor)

2.1.1.8 Willing to use acceptable forms of contraception, if applicable, or abstinence to prevent pregnancy.

Exclusion Criteria

2.1.1.9 Any of the following hematologic abnormalities

2.1.1.9.1 Hemoglobin < 10.0 g/dL

2.1.1.9.2 Neutrophil count < 1500/mm^3

2.1.1.9.3 Platelet count < 75,000/mm^3

2.1.1.9.4 PT or PTT $\geq$ 1.5X ULN (with the exception of patients with known clotting disorders or known lupus anticoagulant).

2.1.1.10 Any of the following hepatic abnormalities

2.1.1.10.1 ALT/SGPT and/or AST/SGOT > 2.5X ULN

2.1.1.10.2 Total bilirubin > 1.5X ULN unless attributable to protease inhibitor therapy.

2.1.1.11 Positive tests (antibody and/or antigen) for hepatitis B and hepatitis C viruses, unless the result is consistent with prior vaccination or prior infection with full recovery.

2.1.1.12 Acute infection requiring therapy at time of enrollment. Participants may be eligible for study after being on stable and appropriate anti-infective therapy.

2.1.1.13 Chemotherapy for active cancer.

2.1.1.14 Documented history of non-adherence to antiretroviral treatment regimen within 12 months of study entry.

2.1.1.15 Pregnancy or breastfeeding.

2.1.1.16 Use of immunosuppressive or immunomodulating agents within 8 weeks of study enrollment. Note: patients receiving oral corticosteroids for management of asthma or contact hypersensitivity for < 14 days in duration will be allowed to enroll as long as it has been > 30 days since oral corticosteroid administration.

2.1.1.17 Known immediate hypersensitivity to yeast or any of the vaccine components.

2.1.1.18 Use of investigational agents within 4 weeks prior to study enrollment.

2.1.1.19 Active external genital warts requiring treatment or CIN2/3

2.1.1.20 Any clinically significant diseases (other than HIV infection) or findings during study screening that, in the opinion of the Principal Investigator or Lead Associate Investigator, may interfere with the study.

2.1.2 Cohort 2 Inclusion and Exclusion Eligibility Criteria

Inclusion Criteria

2.1.2.1 Age 12 to 26 years

2.1.2.2 Females and males

2.1.2.3 HIV-positive

2.1.2.4 CD4 cell count and HIV-1 RNA level parameters

2.1.2.4.1 CD4 cell count $\geq$ 500 cells/mm^3

2.1.2.4.2 HIV-1 RNA level by RT PCR $\leq$ 20,000 copies/ml.

2.1.2.5 *Not* receiving antiretroviral treatment with CD4 and viral load parameters as outlined in 2.1.2.4.

2.1.2.6 Patients $\geq$ 18 years willing to provide informed consent or parent/guardian willing to provide informed consent for minor children <18 years of age.

2.1.2.7 Informed assent for patients 12-17 years of age (Optional at the discretion of the Principal Investigator and Parent/Guardian based on maturity level of minor)

2.1.2.8 Willing to use acceptable forms of contraception, if applicable, or abstinence to prevent pregnancy.

Exclusion Criteria

2.1.2.9 Any of the following hematologic abnormalities:

2.1.2.9.1 Hemoglobin < 10.0 g/dL

2.1.2.9.2 Neutrophil count < 1500/mm^3

2.1.2.9.3 Platelet count < 75,000/mm^3

2.1.2.9.4 PT or PTT $\geq$ 1.5X ULN (with the exception of patients with known clotting disorders or known lupus anticoagulant).

2.1.2.10 Any of the following hepatic abnormalities

2.1.2.10.1 ALT/SGPT and/or AST/SGOT > 2.5X ULN

2.1.2.10.2 Total bilirubin > 1.5X ULN unless attributable to protease inhibitor therapy.

2.1.2.11 Positive tests (antibody and/or antigen) for hepatitis B and hepatitis C viruses, unless the result is consistent with prior vaccination or prior infection with full recovery.

2.1.2.12 Acute infection requiring therapy at time of enrollment. Participants may be eligible for study after being on stable and appropriate anti-infective therapy.

2.1.2.13 Chemotherapy for active cancer.

2.1.2.14 Pregnancy or breastfeeding.

2.1.2.15 Use of immunosuppressive or immunomodulating agents within 8 weeks prior to study enrollment. Note: patients receiving oral corticosteroids for management of asthma or contact hypersensitivity for $\leq$ 14 days in duration will be allowed to enroll as long as it has been $\geq$ 30 days since oral corticosteroid administration.

2.1.2.16 Known immediate hypersensitivity to yeast or any of the vaccine components.

2.1.2.17 Use of investigational agents within 4 weeks prior to study enrollment.

2.1.2.18 Active external genital warts requiring treatment or CIN 2/3.

2.1.2.19 Any clinically significant diseases (other than HIV infection) or findings during study screening that, in the opinion of the Principal Investigator or Lead Associate Investigator may interfere with the study.

2.1.3 Cohort 3 Inclusion and Exclusion Eligibility Criteria

Inclusion Criteria

2.1.3.1 Age 12 to 26 years

2.1.3.2 Females and males

2.1.3.3 HIV-negative

2.1.3.4 Patients $\geq$ 18 years willing to provide informed consent or parent/guardian willing to provide informed consent for minor children <18 years of age.

2.1.3.5 Informed assent for patients 12-17 years of age (Optional at the discretion of the Principal Investigator and Parent/Guardian based on maturity level of minor)

2.1.3.6 Willing to use acceptable forms of contraception, if applicable, or abstinence to prevent pregnancy.

Exclusion Criteria

2.1.3.7 Any of the following hematologic abnormalities:

2.1.3.7.1 Hemoglobin < 10.0 g/dL

2.1.3.7.2 Neutrophil count < 1500/mm^3

2.1.3.7.3 Platelet count < 75,000/mm^3

2.1.3.7.4 PT or PTT $\geq$ 1.5X ULN (with the exception of patients with known clotting disorders or known lupus anticoagulant).

2.1.3.8 Any of the following hepatic abnormalities

2.1.3.8.1 ALT/SGPT and/or AST/SGOT > 2.5X ULN

2.1.3.8.2 Total Bilirubin > 1.5X ULN unless attributable to protease inhibitor therapy.

2.1.3.9 Positive tests (antibody and/or antigen) for HIV, hepatitis B and hepatitis C viruses, unless the result is consistent with prior vaccination or prior infection with full recovery.

2.1.3.10 Acute infection requiring therapy at time of enrollment. Participants may be eligible for study after being on stable and appropriate anti-infective therapy.

2.1.3.11 Chemotherapy for active cancer.

2.1.3.12 Pregnancy or breastfeeding

2.1.3.13 Use of immunosuppressive or immunomodulating agents within 8 weeks prior to study enrollment. Note: patients receiving oral corticosteroids for management of asthma or contact hypersensitivity for $\leq$ 14 days in duration will be allowed to enroll as long as it has been $\geq$ 30 days since oral corticosteroid administration.

2.1.3.14 Known immediate hypersensitivity to yeast or any of the vaccine components.

2.1.3.15 Use of investigational agents within 4 weeks prior to study enrollment.

2.1.3.16 Active external genital warts requiring treatment or CIN 2/3.

2.1.3.17 Any clinically significant diseases or findings during study screening that, in the opinion of the Principal Investigator or Lead Associate Investigator may interfere with the study.

2.2 RESEARCH ELIGIBILITY EVALUATION

Protocol screening evaluation will be performed within 14 days of starting HPV vaccination and will include a complete history, review of systems and physical examination with documentation of abnormalities.

2.2.1 Screening Studies

2.2.1.1 HIV serology

2.2.1.2 Hepatitis B serology: anti-HepB S, HepB S ag

2.2.1.3 Hepatitis C serology: anti-HCV

2.2.1.4 CBC with differential, platelets and reticulocyte count

2.2.1.5 PT, PTT

2.2.1.6 Acute panel

2.2.1.7 Hepatic panel

2.2.1.8 Mineral panel

2.2.1.9 Lipid panel

2.2.1.10 Amylase, lipase

2.2.1.11 Quantitative Immunoglobulins and IgG subclasses

2.2.1.12 Urinalysis

2.2.1.13 VDRL

2.2.1.14 Serum pregnancy test in post-menarchal females

2.2.1.15 EKG

2.2.1.16 Chest x-ray

2.2.1.17 CD4 percent and absolute cell count (for HIV-infected patients)

2.2.1.18 HIV-1 RNA levels by RT PCR (for HIV-infected patients)

2.2.1.19 Gynecologic evaluation, cervical cytology (Pap smear) and HPV DNA testing (performed on sexually active females only)

2.3 PATIENT REGISTRATION AND TREATMENT RANDOMIZATION

This is a non-randomized study. Candidate screening will be carried out using NCI protocol 01-C-0129 "Eligibility Screening and Tissue Procurement for the National Cancer Institute (NCI) Center for Cancer Research (CCR) Clinical Protocols". Following eligibility screening, each patient will be discussed with the Principal Investigator or the Lead Associate Investigator. If they are found to be eligible, the patient and their guardian (if applicable) will be presented with a detailed description of the study protocol plan and treatment. The specific requirements, objectives, risks, alternatives, time commitments and potential benefits will be reviewed with the patient and guardian (if applicable). The Informed Consent (patients $\geq$ 18 years or parent/legal guardian of patients <18 years) and Informed Assent (patients 12-17 years) document(s) will be given to the patient and their guardian (if applicable) which they will be asked to review and ask questions about prior to agreeing to participate in the protocol. The patient will be reassured that participation on this trial is entirely voluntary and that they may with withdraw or decide against receipt of vaccination at any time without adverse consequences. The Principle Investigator or their designee is responsible for obtaining a signed statement of informed consent (and assent if the patient is 12-17 years of age) approved by the IRB. A Confirmation of Eligibility Checklist must also be completed and submitted via FAX (FAX #: 301-480-0757) or by internet/intranet to the Central Registration Office between the hours of 8:30am – 5:00pm, Monday through Friday. No evening weekend or holiday registrations will be permitted. Patients must be registered within 24 hours of signing the consent document. Once eligibility is confirmed and the patient is registered, The Central Registration Office will FAX a confirmation of the registration to the PI and will also confirm the

patient registration with the Clinical Center Investigational Drug Pharmacy. Patient study numbers will be assigned as follows:

Cohort 1 (HIV-infected on HAART): study numbers 101-135

Cohort 2 (HIV-infected, on no therapy): study numbers 201-235

Cohort 3 (HIV-negative controls): study numbers 301-335.

The Central Registration Office will also be notified if a patient is taken off protocol- either due to study withdrawal or study completion. This study does not involve any treatment randomization.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a non-randomized, prospective, phase I study of the quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine. The vaccine is a non-infectious recombinant, vaccine prepared from highly purified VLPs of the major capsid (L1) protein of HPV types 6, 11, 16 and 18.[79] The vaccine was approved and licensed by the FDA for clinical use on June 8, 2006. The study includes 3 cohort populations:

Cohort 1: HIV-infected patients receiving stable HAART therapy

Cohort 2: HIV-infected patients on no antiretroviral therapy

Cohort 3: HIV-negative, healthy, age- and gender-matched controls

The study is designed to determine the safety and immunogenicity of this recombinant vaccine in pre-adolescents, adolescents and young adults with HIV infection who are at increased risk of HPV co-morbidity because of their HIV disease. Since HPV co-morbidity has been documented in both HIV-infected men and women, the vaccine will be administered to both males and females in this study. A cohort of healthy, age-matched control patients will allow comparison of safety and immunogenicity endpoints between HIV-negative and HIV-positive study subjects. The study design will also permit comparison of immunogenicity between HIV-infected patients on HAART and those not receiving antiretroviral treatment. Oral/buccal and anogenital sampling for HPV DNA will be performed on all study subjects to allow cross-sectional characterization of HPV DNA positivity in the study cohort populations. In addition, HPV and HIV knowledge and risk and sexual behaviors will be assessed in all study subjects. Total study duration is 4 years.

Study subjects will be recruited through dissemination of protocol information and eligibility criteria to pediatric and adolescent HIV clinical programs in the Baltimore/D.C. Metropolitan area including the University of Maryland, Johns Hopkins University, Children's National Medical Center, Howard University Hospital, and Georgetown Hospital. In addition to web-based dissemination of information (www.ClinicalTrials.gov) protocol information will be distributed to community-based organizations serving HIV-infected children, adolescents and at-risk youth. Providers will be encouraged to refer HIV-uninfected siblings to enroll in the HIV-negative control cohort of the study. No incentives will be offered for participation in this study.

3.2 DRUG AND VACCINE ADMINISTRATION

3.2.1 Antiretroviral Therapy

Patients in Cohort 1 are required to be on a stable, combination highly active antiretroviral regimen based on current Department of Health and Human Services (DHHS) Guidelines for treatment of HIV infection[98] for ≥ 6 months with CD4 and viral load parameters at entry as specified in the inclusion criteria (2.1.1.5). Whenever possible, changing antiretroviral therapy should be avoided unless needed for optimal patient care. Antiretroviral drugs obtained through expanded access programs are permitted.

3.2.2 Quadrivalent HPV (types 6, 11, 16, 18) Recombinant Vaccine Administration

Quadrivalent HPV vaccine will be administered intramuscularly as 3 separate 0.5ml doses, in single use, prefilled syringes at 0, 2 and 6 months (± 2 weeks) in an outpatient clinic setting. Vaccine should be administered intramuscularly in the deltoid region of the upper arm or in the higher anterolateral area of the thigh. Thorough agitation immediately before administration is necessary to maintain suspension of the vaccine. Following thorough agitation, the vaccine is a white cloudy liquid. The vaccine should not be administered if particulate matter is present or if it appears discolored. Patients will remain under nursing observation for 2 hours following the first injection. Vaccine-related adverse reactions will be evaluated using vaccination report card (VRC)-aided surveillance for 14 days after each dose of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine in all study subjects. Per the ACIP Recommendations for the Use of Quadrivalent HPV Vaccine, the vaccine can be administered at the same visit when other age appropriate vaccines are indicated, such as Tdap, Td and MCV4.[84] Study subjects with minor acute illnesses e.g. upper respiratory tract infections or diarrhea with a temperature < 101°F, will be allowed to receive vaccine. Vaccination will be deferred for individuals with moderate or severe acute illnesses and/or temperature ≥ 101°F until after the illness resolves.

3.3 TREATMENT MODIFICATIONS

3.3.1 Antiretroviral Therapy

Cohort 1 HIV-Infected Patients on HAART

In the event that a *change* in antiretroviral therapy is warranted based on clinical, immunologic (CD4 count) or viral (HIV-1 RNA levels) parameters, regimen changes will be:

- Made after non-adherence to the current treatment regimen has been *ruled out* as a reason for the observed changes in clinical, immunologic or viral parameters warranting the change in therapy.
- Implemented according to principles and recommendations outlined in the DHHS treatment guidelines. [98]
- Managed by the patient's referring physician in consultation with the protocol team.

Patients that require a *change* in their antiretroviral treatment regimen *prior to study month 7* and completion of all three quadrivalent HPV vaccinations will be taken off study and will not count towards the accrual ceiling for Cohort 1. Up to 10 additional patients will be allowed to enroll in Cohort 1 as replacements to reach the intended Cohort 1 N= 35. Patients that require a *change* in their antiretroviral treatment regimen *after study month 7* following completion of all three quadrivalent HPV vaccinations will be allowed to remain on study.

In the event that a *substitution* in antiretroviral therapy is warranted based on antiretroviral drug toxicity, regimen changes will be:

- Implemented according to principles and recommendations outlined in the DHHS treatment guidelines. [98]

Patients that require a *substitution* in their antiretroviral treatment regimen, whether *prior to* or *after* completion of all three quadrivalent HPV vaccinations will be allowed to remain on study.

Cohort 2 HIV-Infected Patients on No Therapy

In the event that *initiation* of antiretroviral therapy is warranted based on clinical, immunologic (CD4 count) or viral (HIV-1 RNA levels) parameters, antiretroviral treatment will be:

- Implemented according to principles and recommendations outlined in the DHHS treatment guidelines. [98]
- Managed by the patient's referring physician in consultation with the protocol team.

Patients that require *initiation* of antiretroviral treatment *prior to study month 7* and completion of all three quadrivalent HPV vaccinations will be taken off study and will not count towards the accrual ceiling for Cohort 2. Up to 10 additional patients will be allowed to enroll in Cohort 2 as replacements to reach the intended Cohort 2 N= 35. Patients that require *initiation* of antiretroviral treatment *after study month 7* following completion of all three quadrivalent HPV vaccinations will be allowed to remain on study.

3.4 PHARMACOKINETIC STUDIES

As this is a vaccine study, no pharmacokinetic studies will be performed.

3.5 ON STUDY EVALUATION (REFER TO APPENDICES 2 AND 3)

All study visits for this protocol will be performed at the NIH Clinical Center in Bethesda, MD.

3.5.1 Vaccine Study Visits: Months 0, 2 and 6 (± 2 weeks)

3.5.1.1 Clinical Evaluation

3.5.1.1.1 Review of systems and documentation of symptoms.

3.5.1.1.2 Specifically document as present or absent: fever ≥ 101°F, malaise, headache, rash, or other signs or symptoms of acute illness.

3.5.1.1.3 Height and weight.

3.5.1.1.4 Vital signs.

3.5.1.1.5 Directed physical examination including skin exam and lymph node survey and documentation of any abnormalities.

3.5.1.1.6 Provision of Vaccination Report Card (VRC) to study subject.

3.5.1.2 Laboratory Evaluation

3.5.1.2.1 CBC with differential

3.5.1.2.2 Acute panel

3.5.1.2.3 Hepatic panel

3.5.1.2.4 Mineral panel

3.5.1.2.5 Serum pregnancy test in post-menarchal females

3.5.1.2.6 CD4 percent and absolute cell count

3.5.1.3 Research Evaluations (**refer to Appendices 5 and 6**)

3.5.1.3.1 HPV DNA Testing (Month 0 only, all subjects) performed by Roche Molecular Diagnostics, Pleasanton, CA 94588.

3.5.1.3.1.1 Cytobrush in PreservCyte (10ml)

3.5.1.3.1.2 External anogenital sampling: Medscand Cytobrush® Plus (CooperSurgical, http://www.coopersurgical.com)

3.5.1.3.1.2.1 Females Brush 1: labia prepuce and external vulva Brush 2: anogenital junction

3.5.1.3.1.2.2 Males Brush 1: glans, coronal sulcus, and scrotum Brush 2: anogenital junction

3.5.1.3.2 Serum for HPV antibody titers performed by Merck (Month 0 only, all subjects. Competitive luminex assay). 10ml RTT

3.5.1.3.3 Studies to be performed by Dr. Ligia Pinto, NCI, Frederick (Month 0 only, all subjects):

3.5.1.3.3.1 Functional HPV antibody neutralization assays.

3.5.1.3.3.2 Cryopreserved PBMCs (6 tubes 10ml sodium heparin GTT) for:

3.5.1.3.3.2.1 HPV & HIV lymphocyte proliferation assays

3.5.1.3.3.2.2 Cytokine induction assay

3.5.1.3.3.2.3 Luminex multi-cytokine profiling of PBMCs

3.5.1.3.4 Extended FACS/Quantitative lymphocyte subpopulation studies: 10ml sodium heparin GTT (Month 0 only, HIV-infected subjects only)

Proposed 5 Color FACS Panel for Naïve & Memory Lymphocyte Subsets & Activation Markers

Tube #	AB1	AB2	AB3	AB4	AB5	Comment
1	CD45	CD14	CD3	CD56	CD19	
2	CD45	CD14	CD3	CD4	CD8	
3	HLA-DR	CD38	CD3	CD4	CD8	Activation CD4, CD8
4	IgG1	CD25	CD3	CD4	CD8	Activation CD4, CD8
5	CD27	CD45RO	CD3	CD4	CD8	Naïve, Central Memory, Effector Memory
Possible Additional Tubes for Subset Analysis						
6	CD45RA	CD31	CD3	CD4	CD8	Differential TREC levels
7	CD45RA	CCR7	CD3	CD62L	CD4	Naïve, Central Memory, Effector Memory*
8	CD45RA	CCR7	CD3	CD62L	CD8	Naïve, Central Memory, Effector Memory*

*Note: Must be performed on fresh PBMCs as CD62L is lost on frozen cells.

3.5.1.3.5 Serum Storage 10ml RTT (Leidos): for L. Pinto functional HPV neutralization antibody assay and for possible later quantitative measurement of HIV-related chemokines MIP-1α, MIP-1β and RANTES

3.5.1.3.6 Whole blood for HPV DNA testing 6ml Dark Lavender K_2EDTA, collect 3 tubes (18ml total), Drs. Harvey Alter & Thomas Zheng (Month 0 only)

3.5.1.3.7 Research assays immunologic responses 10ml sodium heparin GTT, collect 2 tubes (20ml total), Dr. Jay Berzofsky. (Months 0, 2 and 6 only, HIV-infected subjects only)

3.5.1.3.8 Administration of HPV/HIV knowledge and risk and sexual behavior surveys. (Month 0 only. **Refer to Appendices 7-10**)

3.5.2 Follow-Up Study Visits Year 1: Months 1, 3, 7 (± 2 weeks) and 12 (± 30 days)

3.5.2.1 Clinical Evaluation

3.5.2.1.1 Review of systems and documentation of symptoms.

3.5.2.1.2 Height and weight.

3.5.2.1.3 Vital signs.

3.5.2.1.4 Directed physical examination including skin exam and lymph node survey and documentation of any abnormalities.

3.5.2.1.5 Gynecologic evaluation, cervical cytology (Pap smear) and HPV DNA testing (Month 12 only, sexually active females only)

3.5.2.2 Laboratory Evaluation

3.5.2.2.1 CBC with differential

3.5.2.2.2 Acute panel

3.5.2.2.3 Hepatic panel

3.5.2.2.4 Mineral panel

3.5.2.2.5 Lipid panel, amylase/lipase, urinalysis (Month 12 only)

3.5.2.2.6 Quantitative Immunoglobulins and IgG subclasses (Month 12 only)

3.5.2.2.7 Serum pregnancy test in post-menarchal females

3.5.2.2.8 CD4 percent and absolute cell count

3.5.2.2.9 HIV-1 RNA levels (HIV-infected patients only)

3.5.2.3 Research Evaluation

3.5.2.3.1 Serum for HPV antibody titers performed by Merck (Months 7 and 12 only. Competitive luminex assay). 10ml RTT

3.5.2.3.2 Studies to be performed by Dr. Ligia Pinto, NCI, Frederick (all subjects)

3.5.2.3.2.1 Functional HPV antibody neutralization assays.

3.5.2.3.2.2 Cryopreserved PBMCs (6 tubes sodium heparin 10ml GTT) for:

3.5.2.3.2.2.1 HPV & HIV lymphocyte proliferation assays

3.5.2.3.2.2.2 Cytokine induction assay

3.5.2.3.2.2.3 Luminex multi-cytokine profiling of PBMCs

3.5.2.3.3 Extended FACS/Quantitative lymphocyte subpopulation studies: 10ml sodium heparin GTT as outlined in 3.5.1.3.4 (HIV-infected subjects only)

3.5.2.3.4 Serum Storage 10ml RTT (Leidos): for L. Pinto functional HPV neutralization antibody assay and for possible later quantitative measurement of HIV-related chemokines MIP-1α, MIP-1β and RANTES

3.5.2.3.5 Whole blood for HPV DNA testing Dark Lavender K_2 EDTA, collect 3 tubes (18ml total), Drs. Harvey Alter & Thomas Zheng (Months 7 and 12 only)

3.5.3 Follow-Up Study Visits After Year 1: Months 18, 24, 30, 36, 42 and 48 (± 30 days).

3.5.3.1 Clinical Evaluation

3.5.3.1.1 Review of systems and documentation of symptoms.ß

3.5.3.1.2 Height and weight.

3.5.3.1.3 Vital signs.

3.5.3.1.4 Directed physical examination including skin exam and lymph node survey and documentation of any abnormalities.

3.5.3.1.5 Gynecologic evaluation, cervical cytology (Pap smear) and HPV DNA testing (Months 24, 36 and 48; sexually active females only)

3.5.3.2 Laboratory Evaluation

3.5.3.2.1 CBC with differential

3.5.3.2.2 Acute panel

3.5.3.2.3 Hepatic panel

3.5.3.2.4 Mineral panel

3.5.3.2.5 Quantitative Immunoglobulins and IgG subclasses (Months 24, 36 and 48)

3.5.3.2.6 Lipid panel, amylase/lipase, urinalysis (Months 24, 36 and 48)

3.5.3.2.7 Serum pregnancy test in post-menarchal females

3.5.3.2.8 CD4 percent and absolute cell count

3.5.3.2.9 HIV-1 RNA levels (HIV-infected patients only)

3.5.3.3 Research Evaluation

3.5.3.3.1 Serum for HPV antibody titers performed by Merck (Months 24 and 48 only Competitive luminex assay). 10ml RTT

3.5.3.3.2 Studies to be performed by Dr. Ligia Pinto, NCI, Frederick (all subjects; Months 24, 36, 48 only)

3.5.3.3.2.1 Functional HPV antibody neutralization assays.

3.5.3.3.2.2 Cryopreserved PBMCs (6 tubes sodium heparin 10ml GTT) for:

3.5.3.3.2.2.1 HPV & HIV lymphocyte proliferation assays

3.5.3.3.2.2.2 Cytokine induction assay

3.5.3.3.2.2.3 Luminex multi-cytokine profiling of PBMCs

3.5.3.3.3 Extended FACS/Quantitative lymphocyte subpopulation studies: 10ml sodium heparin GTT as outlined in 3.5.1.3.4 (HIV-infected subjects only)

3.5.3.3.4 Serum Storage 10ml RTT (Leidos): for L. Pinto functional HPV neutralization antibody assay and for possible later quantitative measurement of HIV-related chemokines MIP-1α, MIP-1β and RANTES

3.5.3.3.5 Whole blood for HPV DNA testing 6ml Dark Lavender K_2 EDTA, collect 3 tubes (18ml total), Drs. Harvey Alter & Thomas Zheng (Months 24, 36 and 48 only)

3.6 CONCURRENT THERAPIES

3.6.1 Contraindicated Therapies

3.6.1.1 Pharmacologic doses of immune modulating agents including IVIG. Patients requiring oral corticosteroids for management of severe asthma or contact hypersensitivity for $\leq$ 14 days in duration will be allowed to receive them on study. Patients must not have received oral steroids within 14 days prior to receipt of quadrivalent HPV vaccination. Topical steroids, such as creams and lotions, as well as inhaled steroids, are permitted.

3.6.1.2 Cyclophosphamide, hydroxurea, methotrexate or other immunosuppressive drugs.

3.6.1.3 Recombinant cytokines or growth factors

3.6.2 Allowed Therapies

Study subjects will be allowed to take multivitamins, oral contraceptives, and other medications as clinically indicated. Use of herbal or nutritional supplements is discouraged. For females taking oral contraceptives that are also receiving HAART, study investigators will verify that their contraceptives do not interfere with any antiretroviral drugs in their current HAART regimen. In clinical studies of GARDASIL®, use of hormonal contraceptives or lack of use of hormonal contraceptives among study participants did not alter vaccine efficacy.[83] Results from clinical studies also indicate that GARDASIL® may be administered concomitantly (at a separate injection site) with recombinant hepatitis B vaccine. Although co-administration of GARDASIL® with other vaccines has not been studied, ACIP Recommendations state that the vaccine may be given at the same visit when other age appropriate vaccines are provided such as Tdap, Td and MCV4.[84] For HIV-infected study subjects, it is anticipated that there will be no need for HIV-related OI prophylaxis given the high CD4 cell count entry criteria to enroll on study.

3.7 OFF TREATMENT AND OFF STUDY CRITERIA

- Off Treatment- Any Grade III or higher toxicity possibly or probably related to vaccine that does not resolve to baseline within 4 weeks with appropriate intervention.
- Off Treatment- Any Grade IV toxicity unrelated to vaccine that does not resolve to within grade 1 or lower within 8 wks.
- Off Treatment- Severe or life-threatening immediate hypersensitivity reactions following receipt of quadrivalent HPV vaccine.
- Off Treatment and Off Study- Development of an acute life-threatening condition or cancer.
- Off Treatment and Off Study- Inability to adhere to HAART.
- Off Treatment and Off Study- Discontinuation of HAART for >6 wks due to antiretroviral toxicity.
- Off Treatment- > 30% decrease in baseline CD4 cell count or percent or decrease in absolute CD4 count to < 350 cells/mm3 confirmed by at least 2 measurements 4 weeks apart.
- Off Treatment- Increase in HIV-1 RNA levels to > 100,000 copies/ml confirmed by at least 2 measurements 4 weeks apart.
- Off Treatment- Pregnancy. Patients will be taken off-study and advised to defer completion of their vaccination regimen until resolution of the pregnancy. Patients will also be reported to the Merck Pregnancy Registry (800-986-8999) that monitors fetal outcomes of pregnant women exposed to GARDASIL®.
- Off Study- Non-compliance with study protocol evaluations and visits.
- Off Study- Study discontinuation at the request of the patient or at the discretion of the Principal Investigator or Lead Associate Investigator if they determine that study withdrawal is in the patient's best interest.

3.8 POST TREATMENT EVALUATION / FOLLOW-UP

This study is 4 years in duration. At the final, month 48, study end-point, study participants will be 42 months out from completion of their vaccination series and no further follow-up or post study evaluations will be performed. For those subjects who meet off-study criteria prior to study completion, the following evaluations will be performed within 8 weeks of being taken off-study.

3.8.1 Clinical Evaluation

3.8.1.1 Comprehensive review of systems and directed physical examination with documentation of abnormalities.

3.8.2 Laboratory Evaluation

3.8.2.1 CBC with differential, platelet count

3.8.2.2 PT, PTT

3.8.2.3 Acute panel

3.8.2.4 Hepatic panel

3.8.2.5 Mineral panel

3.8.2.6 Lipid panel

3.8.2.7 Amylase, lipase

3.8.2.8 Urinalysis

3.8.2.9 Quantitative Immunoglobulins and IgG subclasses

3.8.2.10 CD4 percent and absolute cell count

3.8.2.11 HIV-1 RNA levels by RT PCR (for HIV-infected patients)

3.8.3 Optional Research Evaluation

3.8.3.1 Serum for HPV antibody titers performed by Merck (competitive luminex assay).

3.8.3.2 Quantitative lymphocyte subpopulation studies as outlined in 3.5.1.3.4 (HIV-infected study subjects only).

3.8.3.3 Serum for storage (Leidos) for possible later quantitative measurement of HIV-related chemokines MIP-1α, MIP-1β and RANTES.

3.8.3.4 Whole blood for HPV DNA testing 6ml PT EDTA, collect 3 tubes (18ml total), Drs. Harvey Alter & Thomas Zheng.

Additional follow-up may be conducted at the discretion of the Principal Investigator or Lead Associate Investigator if study withdrawal was due to toxicity and additional monitoring is warranted until satisfactory resolution is achieved.

4 SUPPORTIVE CARE

4.1 ANTIRETROVIRAL THERAPY

Appropriate evaluation and supportive care for toxicity, intolerance, hypersensitivity or other clinical adverse events related to antiretroviral therapy (for those receiving it) will be made according to standard of care.

4.2 VACCINE-RELATED EVENTS

Appropriate evaluation of and supportive care for immediate hypersensitivity reactions as well as delayed adverse reactions to quadrivalent HPV vaccination will be made according to standard care.

Study subjects that have objective of immediate hypersensitivity to the quadrivalent HPV vaccine or any of its components will not receive further doses of vaccine.

4.3 CYTOLOGIC SCREENING AND MANAGEMENT OF ABNORMAL CERVICAL CYTOLOGIC FINDINGS

All sexually active female study subjects will undergo routine gynecologic evaluation, Pap smear testing (includes endocervix, exocervix and vaginal sampling) and HPV DNA testing. Due to the increased prevalence of cytologic abnormalities and SIL in women with HIV, sexually active, HIV-infected female study subjects will undergo comprehensive gynecologic evaluation, Pap smear and HPV DNA testing *annually* for the duration of the study, as recommended per CDC STD and HPV screening guidelines.[74] Sexually active, healthy, HIV-negative female control subjects will undergo cervical cancer screening according to published guidelines: American Cancer Society,[100] U.S. Preventive Services Task Force[101] and the American College of Obstetricians and Gynecologists.[102] Study subjects found to have abnormal cytology (ASCUS, LSIL including CIN1, or HSIL including carcinoma in situ, CIN2 and 3) will be managed according to Clinical Management Guidelines for Management of Abnormal Cytology and Histology[99] and in consultation with Dr. Pamela Stratton, Chief, CC Gynecology Consult Service.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system C3D and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for 30 days after removal from study treatment or until off-study, whichever comes first.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

5.1.1 Study Summaries

Study summaries will be generated every 6 months and forwarded to the FDA and Merck, which is providing study support, in addition to the annual reporting requirements outlined in section **7.4.2**.

5.2 RESPONSE CRITERIA

The primary focus of this clinical study is immunogenicity as measured by development of and change in antibody titers to the four HPV types within the quadrivalent HPV vaccine. HPV-specific antibody titers will be measured at baseline and s/p receipt of 1, 2 and 3 doses of vaccine, in addition to serial, longitudinal monitoring of HPV titers every 6 months following completion of the vaccination series for a total of 4 years to assess antibody titer decay and duration of immunogenicity.

The only clinical outcomes that will be monitored for response are for safety criteria, as this study is not statistically powered to assess vaccine efficacy even on a preliminary level in the currently defined study cohort populations. Safety will be monitored through the use of Vaccination Report Card (VRC) Surveillance for Adverse Vaccine Experiences to assess local injection site and systemic reactions to vaccination, in addition to monitoring of laboratory values. In HIV-infected study subjects, this safety assessment also includes virologic parameters to ensure that HPV VLP vaccination is not associated with upregulation of viral replication and increased HIV-1 RNA levels.

Research assessments that will be evaluated in a descriptive observational manner include:

- Cross-sectional characterization of HPV DNA positivity by oral/buccal and anogenital swabs at baseline in the study cohort populations.
- Longitudinal changes following vaccination in:
  - lymphocyte proliferation assays to HPV and HIV antigens
  - cytokine induction and profile
  - activation and proportional representation of lymphocyte subset populations (HIV-infected patients only)
- Cross-sectional and longitudinal characterization of HPV and HIV knowledge and risk and sexual behaviors in the study cohort populations.

Specific statistical approaches to the primary immunogenicity and virologic safety analyses, and secondary analyses of HIV-related parameters (use of HAART, CD4 cell count and HIV-1 RNA levels) on vaccine immunogenicity are outlined in complete detail in the statistical section 5.4.

5.3 TOXICITY CRITERIA

This study will utilize NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0, published June10, 2003, for toxicity and adverse event monitoring and reporting. (Refer to: http://ctep.info.nih.gov/reporting/ctc_v30.html). All clinical treatment areas will have access to a copy of CTCAE version 3.0.

6 STATISTICAL SECTION

Primary analyses will be for immunogenicity: fold increase in HPV titer following vaccination and for safety.

6.1 PRIMARY IMMUNOGENICITY ANALYSIS PART I

Does vaccine-induced antibody titer differ between study cohorts at a pre-determined primary timepoint X?

4 comparisons:

- Cohort 1 (HIV-infected, on HAART) vs. cohort 2 (HIV-infected, *no* HAART)
- Cohort 1 (HIV-infected, on HAART) vs. cohort 3 (healthy, HIV negative controls)
- Cohort 2 (HIV-infected, *no* HAART) vs. cohort 3 (healthy, HIV negative controls)
- Cohorts 1 & 2 (HIV-infected) vs. cohort 3 (healthy, HIV negative controls)

For each pairwise comparison, allowing for 4 primary comparisons as defined above among cohorts at a single time-point, we will need to plan to test with as small as a 0.0125 p value for each of the 4 comparisons in the primary analysis. With 35 vaccine recipients in each of Cohorts 1, 2 and 3, there will be at least 94% power to detect differences among cohorts as described above, of 1.0 standard deviation effect size for a single time-point.

6.2 PRIMARY IMMUNOGENICITY ANALYSIS PART II

What is the change in vaccine induced antibody titer between baseline and timepoint X?

Timepoints for Analysis:

Timepoint 1 Baseline vs. Month 7 (1mos s/p 3 vaccinations)

Timepoint 2 Baseline vs. Month 12 (6mos s/p 3 vaccinations)

Timepoint 3 Baseline vs. Month 24 (18mos s/p 3 vaccinations)

Timepoint 4 Baseline vs. Month 48 (42mos s/p 3 vaccinations)

Comparisons between baseline and each of the 4 time-points noted above will be made for each of the three defined cohorts of patients receiving vaccine. As there will be 12 paired comparisons performed, the individual tests will be performed using a 0.004 alpha level to adjust for multiple comparisons (0.05/(3x4) =0.0042, which rounds to 0.004). With 35 patients in each of Cohorts 1, 2 and 3 there will be 90% power to detect a change with an effect size equal to 0.75 SD from baseline to each time-point using a 0.004 alpha level two-tailed test, and 99% power to detect a change with a 1 SD effect size from baseline, again using a 0.004 alpha level two-tailed test.

In addition, based upon a 0.003 alpha level two-tailed test, and 1 SD effect size with 4 comparisons between cohorts at N = 4 primary time-points as outlined above, 35 patients receiving vaccine in each of cohorts 1, 2, and 3 will have the following characteristics for the comparisons: Cohorts 1 and 2, or 1 and 3, or 2 and 3 can each be compared with 85% power. In addition, the comparison of Cohorts 1 and 2 vs. 3 will have 96% power with the combined total of 70 patients vs. 35 patients in Cohort 3. The stringent 0.003 alpha level was selected to adjust according to Bonferroni principles: 0.05/(4 comparisons x 4 time-points)=0.05/16=0.0031, or about 0.003).

Finally, in addition to comparisons at each time point, a linear mixed model approach may be used to perform an evaluation of the longitudinal patterns of change. This analysis may also be able to account

for any patients who are unable to be included at each time point, should that be necessary. For both part I and part II primary immunogenicity analyses, seropositivity at baseline for one of the HPV vaccine types will exclude the subject from inclusion in the analysis for that HPV type. If initial cross-sectional evaluation of HPV serostatus among study subjects within each cohort reveals a significant number of subjects with baseline seropositivity to one or more vaccine HPV types resulting in their exclusion from the immunogenicity analysis, expanded enrollment within a cohort may be necessary to ensure the validity of the statistical analyses that have been proposed.

6.3 PRIMARY VIROLOGIC SAFETY ANALYSIS

Are HIV-1 RNA levels equivalent among HIV-infected patients on HAART versus those not on HAART following vaccination between baseline and timepoint X?

The statistical analysis assumes that an equivalence test will be used, and that no more than 5% of HIV-infected individuals would experience a $\geq$ 0.5 $\log_{10}$ (the range of biologic variation) increase in HIV-1 RNA levels within a week following vaccination or at subsequent time-points. The primary goal of this statistical safety analysis is to determine whether the fraction of patients experiencing a $\geq$ 0.5 $\log_{10}$ increase in HIV-1 RNA levels *at any time within the first week* and up to 1 year following the first immunization, may be considered equivalent between those HIV-infected individuals receiving HAART and those not on HAART following receipt of quadrivalent HPV vaccine. Time-point 1 comparisons of changes in the peak HIV-RNA levels from day 1 between Cohort 1 and 2 during any of the evaluations during the first week (see below) will be considered as the most important primary evaluation for this equivalence study and will be the basis for sample size determination. Another primary evaluation will be based on determining the equivalence of the fractions with day 7 vs. day 1 levels $\geq$ 0.5 $\log_{10}$ (the range of biologic variation) increase in HIV-1 RNA levels between the two cohorts.

Equivalence tests can require a tremendous number of subjects in order to allow only a small interval of difference to be interpreted as equivalent. In order to allow for the test to be done with a moderate number of patients, the two vaccine cohorts will be considered equivalent if the fraction of vaccine recipients in one cohort who have an HIV-1 RNA level increase $\geq$ 0.5 $\log_{10}$ is within 10% of that of the other cohort. Thirty-five patients are required per arm within each HIV-infected cohort, to have 73% power to document this level of equivalence. Formally stated, 35 patients per arm was selected as the sample size in order to allow a single two-group t-test of proportions with a one-sided 0.10 significance level to have 73% power to reject the null hypothesis that the two cohorts are not equivalent with respect to the fraction of patients experiencing a $\geq$ 0.5 $\log_{10}$ increase in HIV-1 RNA levels *at each individual time comparison--the maximum increase within the first week* and up to 1 year following the first immunization.

Timepoints for HIV-1 RNA Level Analysis: Comparison between Recipients in HIV-Infected Cohorts 1 and 2:

Timepoint 1 Day 1 vs. Day 3 or Day 7 (immediately s/p vaccination) *(primary)*

Timepoint 2 Day 1 vs. mos 1 (1mos s/p 1 vaccination)

Timepoint 3 Day 1 vs. mos 3 (1mos s/p 2 vaccinations)

Timepoint 4 Day 1 vs. mos 7 (1mos s/p 3 vaccinations)

Timepoint 5 Day 1 vs. mos 12 (6mos s/p 3 vaccinations)

Within each cohort, the change in $\log_{10}$ HIV-1 RNA levels will be determined between day 1 and day 7 and will be evaluated to see if the values at the two time points are not statistically different from one another. HIV-1 RNA levels below the lower limits of detection (50 copies/ml) will be expressed as 1.69 $\log_{10}$ on a logarithmic scale equivalent to 49 copies/ml. With a minimum of 27 patients in each cohort of HIV-infected individuals, there is at least 95% power to detect a change from day 1 to day 7 with an effect size of 1 SD and a 0.025 two-sided alpha level test (0.05/2cohorts) after allowing for a Bonferroni adjustment.

In addition, as a secondary evaluation, comparison of changes in HIV-1 RNA levels will also be done among the two cohorts from day 1 to day 7, and months 1, 3, 7 and 12 in order to assess if the change in level may have some association with the cohort in which the patient is enrolled. This evaluation will be considered exploratory for all comparisons and timepoints.

6.4 SECONDARY ANALYSES

Examination of fold increase in HPV titer and correlation with baseline CD4 count and viral load will be treated as a secondary analysis that will primarily be descriptive/exploratory. With 35 vaccine recipients within each cohort, there will be approximately 87% power to detect between a 0.80 correlation coefficient and a 0.50 correlation coefficient with a 2-sided .05 alpha level test.

6.5 ADDITIONAL STATISTICAL ANALYSES BY MERCK

Biostatisticians from Merck will assess geometric mean titers at months 1, 3, 7 (±2 weeks), 12, 24, 36, and 48 (± 30 days) for HIV-infected Cohorts 1 and 2 individually and collectively, as well as HIV-negative Cohort 3, in comparison with geometric mean titers at these timepoints documented in vaccine recipients in their clinical approval and licensing studies.

6.6 DESCRIPTIVE ANALYSES

Analyses of HPV/HIV knowledge and risk behavior survey responses will be descriptive and exploratory for all study cohorts. No statistical comparisons between cohorts are planned. . Study investigators will not have access to raw survey response data and will not be able to link survey responses to study participants.

6.7 ACCRUAL STOPPING RULE

Accrual to the HIV-infected cohorts (Cohort 1 and Cohort 2) will be halted if 3 or more patients within a given cohort or 5 or more total patients between the two cohorts are removed from the study before month 12 for a decline in CD4 count (3.9.7) and/or an increase in HIV-1 RNA levels (3.9.8) as outlined in section 3.9 Off-Treatment/Off-Study Criteria. Occurrence of these events prior to study month 12 would be suggestive of potential excess virologic safety risk possibly related to HPV vaccination, while their occurrence after study month 12 is most likely to be due to the emergence of antiretroviral resistance (Cohort 1 on HAART) or the natural history of the disease within an individual (Cohort 2 on no treatment). Study month 12 was the cutoff chosen for this stopping rule as it includes 12 months of observation following the first HPV vaccination and 6 months of observation following completion of the vaccination series (3 vaccine doses) at study month 6. In the event that accrual to the study is halted, the IRB will be consulted to determine whether the halt on enrollment should be permanent or can be addressed through an amendment to the protocol.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-compliance (NIH Definition)

7.1.10 The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 RELATIONSHIP TO THERAPY

The Principal Investigator will assess whether the AE or SAE is associated with the administration of quadrivalent HPV (Types 6, 11, 16, 18) recombinant vaccine:

- "Related" includes, possible, probably, and definitely related events.
- "Not related" includes unlikely and not related.

7.3 NIH INTRAMURAL IRB AND CLINICAL DIRECTOR (CD) REPORTING

7.3.1 NIH Intramural IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NIH Intramural IRB and NCI CD:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received by within 7 days of PI awareness via iRIS.

7.3.2 NIH Intramural IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NIH Intramural IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;
   - All Serious Events regardless of attribution.

   NOTE: Grade 1 events are not required to be reported.

7.3.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.4 FDA REPORTING CRITERIA

7.4.1 IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

7.4.1.1 Expedited reporting to the FDA

The Sponsor will notify FDA via phone, fax, or email of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information. This will be followed with a written report within 15 days using the MedWatch Form 3500a.
The study Sponsor will notify FDA in writing of any suspected adverse reaction that is both serious and unexpected as soon as possible but no later than 15 calendar days after initial receipt of the information using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.

- The study Sponsor will also report expeditiously as above:
  any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug
- clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure.

7.4.2 FDA Annual Reports (Refer to 21 CFR 312.33)

The study Sponsor will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect as indicated in 21CFR 312.33, and any associated FDA correspondences regarding the IND annual report. The Annual Report will include:

- A summary of the status of the trial, including the title of the study, protocol number, purpose (objectives), a description of the patient population, and a statement as to whether the study has been completed or is ongoing.

- The accrual ceiling and the number enrolled, tabulated by age group, gender, and race. The number whose participation in the study was completed as planned and the number who withdrew from the study for any reason.
- Once the study is completed, a brief description of the results should be reported.
- A narrative or tabular summary of the most frequent and most serious adverse experiences by body system.
- A summary of all IND Safety Reports
- A list of subjects who died during participation with the cause of death for each subject.
- A list of subjects who withdrew prematurely during the course of the study in association with any adverse experience, whether or not it was thought to be associated to GARDASIL®
- A description of any protocol modifications during the past year if not previously reported.
- A description of the general investigation plan for the coming year.

7.4.3 Expedited Adverse Event Reporting Criteria to the IND Manufacturer

All SAEs will also be reported to the Merck at the same time as FDA notification. SAE reporting to Merck will be sent to:

Linda Hostelley, Vice President
Worldwide Product Safety & Quality Assurance
Merck & Co., Inc.
P.O. Box 4
West Point, PA 19486
Phone: (215) 652-8071
Fax: (215) 993-1220
Linda_Hostelley@Merck.com

7.4.4 Adverse Vaccine Reactions

Adverse vaccine reactions will be reported to the FDA, the IRB and Merck, as well as to the U.S. Department of Health and Human Services Vaccine Adverse Event Reporting System (VAERS): 1-800-822-7967 or on line at www.vaers.hhs.gov. These will be reported within 10 working days. If the event is a SAE or a previously unknown toxicity, it will be reported to the IRB, Merck and the FDA as outlined above. All first occurrences of adverse reactions judged to be related to quadrivalent HPV vaccine will be considered unexpected reactions for the purpose of reporting.

7.5 DATA AND SAFETY MONITORING PLAN

7.5.1 Principal Investigator/Research Team

This phase I, prospective study will be monitored by the Principal Investigator and as required by the FDA as part of the Sponsor Investigator IND held by the Principal Investigator. Based on the minimal level of risk and the number of subjects to be studied, monitoring by a single independent monitor or a data and safety monitoring board (DSMB) is not warranted. Specifically, the protocol is investigating the use of a FDA approved, licensed vaccine in a unique population and vaccine immunogenicity rather than safety is likely to be the most critical outcome finding of this study, particularly given the provisional recommendations of the ACIP for the Use of Quadrivalent HPV Vaccine.[84]

Clinical, laboratory and adverse event data will be monitored a minimum of once monthly or more frequently as accrual to the study increases. From an adverse event, risk and safety-reporting standpoint, the study will be monitored for:

- Excess adverse or vaccine-related events observed in study subjects compared to events reported in clinical trials involving vaccine and placebo subjects.
- *Novel* adverse or vaccine-related events not previously reported in clinical licensing trials of the vaccine.
- Evidence of increased viral replication as measured by serial HIV-1 RNA levels (Days 1,3 and 7) following the first quadrivalent HPV vaccination in HIV-infected individuals.

Clinical research team and medical staff will notify the Principal Investigator of any suspected SAEs with subsequent reporting as previously outlined in 5.3.1.3. The study research nurse coordinator and/or data manager will generate a bi-weekly (or weekly) report of all on-study laboratory data with correspondent toxicity grading according to CTCAE v3.0 as well as a summary of all clinical and Vaccine Report Card (VRC) surveillance adverse event data for review by The Principal Investigator, the Lead Associate Investigator or their designee.

7.5.2 Sponsor Monitoring Plan

NOTE: As the IND is withdrawn/no longer active, Sponsor monitoring activities are no longer ongoing. This trial will be monitored by personnel employed by a CCR contractor to the NCI, NIH. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

At least 25% of enrolled patients' will be randomly selected and monitored at least quarterly, based on accrual rate. The patients selected will have 100% source document verification done. Additional monitoring activities will include: adherence to protocol specified study eligibility, treatment plans, data collection for safety and efficacy, reporting and time frames of adverse events to the IRB and FDA, and informed consent requirements. Written reports will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 HUMAN SUBJECTS PROTECTIONS

8.1 RATIONALE FOR SUBJECT SELECTION

8.1.1 General

As outlined in section 1.2.6 this study seeks to enroll subjects at risk for acquisition of HPV infection (pre-adolescents, adolescents and young adults 12-26 years of age) and at risk for excess HPV-related morbidity once infected i.e. HIV-infected males and females. Vaccine immunogenicity will be studied in HIV-infected individuals with identical CD4 and viral load parameters at entry who are on HAART (Cohort 1) and not receiving HAART (Cohort 2). The latter cohort will allow assessment of quadrivalent HPV recombinant vaccine immunogenicity and safety in a population similar to those found in developing countries with little or no access to antiretroviral treatment where there is also a concomitant, high burden of prevalent HPV and HIV disease. Both HIV-infected cohorts will permit assessment of whether HPV vaccination is associated with alterations in HIV-1 RNA levels and the impact of CD4 count and viral load on vaccine immunogenicity. In addition, comparison between cohorts will allow assessment of the impact of HAART on vaccine immunogenicity.

8.1.2 Age Exclusion

Although ACIP Recommendations for the use of quadrivalent HPV vaccine recommend vaccination for females 11-12 years of age and the vaccination series can be started in females as young as 9 years of age,[84] children < 12 years of age will not be allowed to participate in this study. Another study investigating the safety and immune response of this vaccine is being conducted in HIV-infected children (both males and females) 7-12 years of age by the IMPAACT (formerly PACTG) Clinical Trials Network: ClinicalTrials.gov Identified: NCT00339040, Myron J. Levin, M.D., Study Chair. The data from our study will complement the data obtained from the IMPAACT study to provide a comprehensive overview of quadrivalent HPV vaccine immunogenicity in the HIV-infected pediatric/adolescent/young adult population at risk for HPV disease. Young adults older than 26 years of age will not be allowed to enroll in this study as the safety and efficacy of quadrivalent HPV vaccine have not been evaluated in adults above the age of 26 years.

8.1.3 Racial & Gender Inclusion

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria outlined in section 2.0. Both males and females are included in this study because of the well-documented risk of HPV acquisition following the initiation of sexual activity during adolescence and young adulthood. HIV disease disproportionately affects racial and ethnic minorities in both the pediatric and adult populations and efforts will be directed to ensure that study accrual is representative of this epidemiologic reality.

8.2 PARTICIPATION OF CHILDREN

Children age ≥ 12 years will be allowed to participate in this study if they are able to give assent and if their parent or guardian gives informed consent. Informed Assent is optional at the discretion of the Principal Investigator and Parent/Guardian based on the maturity level of the minor. Adolescents represent one of the highest-risk groups for acquisition of both HPV *and* HIV infection. Adolescents who acquired HIV-infection early in life via perinatal transmission or through transfusion are especially at risk for co-morbidity due to HPV infection once acquired, as a result of prolonged immunosuppression, lifelong immune dysregulation and because they are also more likely to be heavily treatment-experienced and have limited effective antiretroviral treatment options to control their HIV disease. Given the well-documented immunogenicity and efficacy of the quadrivalent HPV vaccine, this study population, especially those who have not yet become sexually active, has the greatest potential to gain protection from the co-morbidity of HPV disease.

8.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Potential benefits to study participants as a consequence of this research is that vaccination with quadrivalent HPV vaccine may induce immune responses i.e. neutralizing antibody titers that are comparable to those that have been demonstrated to be associated with prevention of genital warts, precancerous or dysplastic lesions (AIS, CIN 2/3, VIN 2/3, VaIN 2/3 and CIN 1) and cervical cancer caused by HPV types 6, 11, 16 and 18. In healthy, HIV-negative women, the vaccine was demonstrated to be highly (100%) efficacious in preventing the pre-neoplastic and neoplastic conditions listed above.[83] In addition, individuals who were already infected with 1 or more vaccine-related HPV types prior to vaccination, were protected from clinical disease caused by the remaining HPV types, suggesting there is potential for protective benefit even in those individuals who are sexually active and have already been exposed to or acquired HPV. This provides an even stronger benefit argument in this population that is uniquely predisposed to excess co-morbidity due to HPV disease. On the other hand, it is

possible that the quadrivalent HPV vaccine will not be as immunogenic in the HIV-infected individuals or if it is immunogenic, the magnitude and duration of the response may not be as sustained as has been observed in those without HIV-infection. Whether or not individuals receive direct benefit from participating in this study, there is indirect benefit to be gained for the HIV-infected population in general from determining vaccine immunogenicity and identifying whether additional primary or boosting doses of vaccine may be necessary to achieve antibody titers levels that have been shown to be protective for acquisition of HPV in HIV negative, immunocompetent populations.

Potential risks include unknown or excessive adverse events related to the safety of the vaccine. This is unlikely to occur because the vaccine is not comprised of live, attenuated or inactivated viruses but rather of highly purified, recombinant virus-like particles (VLPs) of the major capsid (L1) protein of HPV types 6, 11, 16 and 18.[79] In addition, the FDA did not require the pharmaceutical manufacturer to present evidence of safety in immunocompromised populations as a condition for vaccine approval and licensure, and current ACIP Recommendations for use of the vaccine endorse its use in immunocompromised females, with the caveat that the immune response to vaccination and vaccine effectiveness might be less than in females who are immunocompetent.[84] Another potential risk is the upregulation of HIV viral replication associated with vaccine administration. Although likely to be minimized by HAART, it could potentially be more of an issue in those receiving no antiretroviral therapy. Entry criteria parameters were established such that if increases in viral replication were to be observed associated with vaccination, it would be in individuals with HIV-1 RNA levels $\leq$ 20,000 copies/ml at entry with no indication for initiation of antiretroviral therapy i.e. HIV-1 RNA levels $\geq$ 100,000 that are associated with higher risk for disease progression.[85]

8.4 RISKS/BENEFIT ANALYSIS

There are two levels of potential benefit from this protocol. One level is that direct patient benefit may occur as a result of induction of HPV-specific immunity associated with administration of quadrivalent HPV vaccine, that could in turn result in protection from acquisition of HPV infection or establishment of pre-neoplastic and neoplastic HPV-related conditions. The other level involves indirect benefit as a consequence of obtaining increased knowledge regarding vaccine-induced HPV-specific responses in HIV-infected individuals that fall within the approved age range for vaccination. This knowledge may be valuable in ensuring optimal use of the vaccine, particularly in resource poor settings where there is an excess burden of both HIV and HPV disease.

The likelihood of direct benefit for healthy, HIV negative control subjects (Cohort 3) participating in this study is high and their accompanying risk for serious, vaccine-related adverse events is low based on well-established data as detailed in sections 1.2.5 and 8.1.6, respectively. The secondary objectives of the protocol involving this cohort population that address HPV DNA carriage in oral/buccal and anogenital sites as well as characterization of risk behaviors and HPV/HIV knowledge assessment, pose minimal risk to these subjects. In addition, study participants (or their parents) in all cohorts have the opportunity to decline participation as it relates to these specific secondary objectives, without jeopardizing their overall protocol participation.

The likelihood of direct benefit in HIV-infected individuals (Cohorts 1 and 2) is simply *unknown* as no data currently exists regarding HPV vaccine immunogenicity. Their risk of serious, vaccine-related adverse events is also considered to be low as the quadrivalent HPV vaccine is a recombinant protein vaccine and hence has none of the inherent, well-documented increased risks associated with live-attenuated viral vaccines in immuncompromised patients. However, it is unknown whether HPV vaccination will result in increased viral replication. The likelihood of this occurring is extremely low in

individuals receiving HAART (Cohort 1), although in HIV-infected patients on no antiretroviral treatment (Cohort 2), the potential for increased viral replication must be weighed against the likelihood of HPV acquisition and its almost guaranteed co-morbidity in individuals with HIV-infection. Importantly, routine vaccination of HIV-infected individuals is recommended independent of their antiretroviral treatment status and HIV-infected children can receive MMR and varicella vaccines (both live vaccines) if their CD4 lymphocyte count is $\geq$ 15%.[90]

8.5 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

All patients age 18 and over, and the parent/legal guardian of patients age 12-17 will read and sign the informed consent document prior to study enrollment. Members of the protocol team will describe the study, alternative therapies, and the risks and benefits of each to the individual signing the consent. In addition, optional assent will be obtained from adolescent patients/minors of sufficient maturity and their signature at the end of NIH form 2514-1 will document such assent. If adolescent patients express a reluctance to participate, they will be referred to a non-research setting for their care and to receive the HPV vaccine at their discretion in consultation with their local physician.

9 BIOSPECIMEN COLLECTION

The Clinical Support Laboratory (CSL), Leidos, processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. The laboratory is located in a controlled access building and laboratory doors are kept locked at all times. Upon specimen receipt each sample is assigned a unique, sequential CSL accession ID number. All products generated by the protocol that will be stored at the NCI Frederick central repository facility are identified by this accession ID number. An electronic database is used to store information related to patient samples processed by the CSL. Vial labels do not contain any personal identifier information. Samples are stored in inventoried locked laboratory freezers and are routinely transferred to the NCI-Frederick repository facilities for long-term storage. These facilities are operated by Fisher Bioservices, Inc. under subcontract to Leidos. Access to stored clinical samples is restricted. Investigators establish sample collections under "Source Codes" and the investigator responsible for the collections, typically the protocol Principal Investigator, specifies who has access to the collection. Blood samples collected during the course of this research study will be stored for future use only if the patient consents.

When requests are submitted by an NCI investigator for shipment of samples outside of the NIH it is the policy of the CSL to request documentation that a Material Transfer Agreement is in place that covers the specimen transfer. The laboratory does not provide patient identifier information as part of the transfer process but may, at the discretion of the NCI investigator, group samples from individual patients when it is critical to the testing process. The NCI investigator responsible for the sample collection is responsible for ensuring appropriate IRB approvals are in place and that a Material Transfer Agreement has been executed prior to requesting the laboratory to ship samples outside of the NIH.

Blood and tissue specimens collected during the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered in the consent document and the proposed research has undergone prospective IRB review and approval. If new risks are associated with the research (e.g. analysis of germ line genetic mutations) the Principal Investigator must amend the protocol and obtain informed consent from all research subjects.

Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples provided they have an IRB approved protocol and patient consent.

Samples, and associated data, will be stored permanently at the NCI Frederick CSL unless the patient withdraws consent. The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. The CSL will report any freezer problems, lost samples or other problems associated with samples to the IRB, the NCI Clinical Director, and the Office of the CCR, NCI.

10 COLLABORATIVE AGREEMENTS

10.1 CLINICAL TRIALS AGREEMENT (CTA)

A clinical trials agreement, CTA # 697-07, executed on 11/20/08 is in place between the NCI Center for Cancer Research and Merck and Company, Inc. in order to allow for the measurement of serum antibody titers as described in section **3.5.1.3.2**.

10.2 MATERIALS TRANSFER AGREEMENT (MTA)

A materials transfer agreement, MTA # 35922-13 was executed on 5/12/14 between the NCI Center for Cancer Research and Roche Molecular Systems in order to allow for HPV genotyping as described in section **3.5.1.3.1**.

11 PHARMACEUTICAL INFORMATION

11.1 QUADRIVALENT HUMAN PAPILLOMAVIRUS (TYPES 6, 11, 16, 18) RECOMBINANT VACCINE

The *use* of quadrivalent HPV vaccine is considered investigational in the HIV-infected cohort populations being recruited for this study, even though the vaccine is a licensed, approved, *non*-investigational biologic product. As a consequence, the Principal Investigator has filed for an individual Sponsor/Investigator held IND for this and other studies of quadrivalent HPV vaccine in immunocompromised patients.

Merck Research Labs of West Point, PA is the supplier of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine used in this study.

11.1.1 Administration Procedure

Quadrivalent HPV vaccine should be administered intramuscularly in the deltoid region of the upper arm or in the higher anterolateral area of the thigh as 3 separate 0.5ml doses according to the following schedule: first dose at elected date; second dose 2 months after the first dose (±2 weeks); third dose 6 months (±2 weeks) after the first dose. Subcutaneous and intradermal administration have not been studied and are therefore are not recommended.

The vaccine should be used as supplied; no dilution or reconstitution is necessary. The full recommended dose of the vaccine should be used. Shake well before use. Thorough agitation immediately before administration is necessary to maintain suspension of the vaccine. After thorough agitation, the vaccine is a white, cloudy liquid. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Do not use the product if particulates are present or if it appears discolored.

As with all injectable vaccines, appropriate medical treatment should always be readily available in case of rare anaphylactic reactions following the administration of the vaccine.

The decision to administer or delay vaccination because of a current or recent febrile illness depends largely on the severity of the symptoms and their etiology. ***Low-grade fever itself and mild upper respiratory infection are not generally contraindications to vaccination.***

11.1.2 Formulation and Preparation

Quadrivalent HPV vaccine is a non-infectious recombinant vaccine prepared from the highly purified virus-like particles (VLPs) of the major capsid (L1) protein of HPV types 6, 11, 16 and 18.[79] It was approved and licensed by the FDA for clinical use on June 8, 2006 and is marketed by its producer Merck & Co., under the trade name GARDASIL®. The L1 proteins are produced by separate fermentations in recombinant *Saccharomyces cerevisiae* and self-assembled into VLPs. Each 0.5ml vaccine dose contains 20, 40, 40 and 20 mcg of HPV 6, 11, 16, and 18 L1 VLPs, respectively, co-formulated with 225 mcg of Merck's proprietary aluminum hydroxy sulfate adjuvant.

Quadrivalent HPV vaccine is supplied as a 0.5ml single-dose vial (available in a carton of ten) or as a 0.5ml single-dose prefilled syringe (available in a carton of six). Prefilled syringes are preassembled with Ultra Safe® Passive™ Needle Guard devices. A one-inch, 25-gauge needle is provided separately in the package.

11.1.3 Incompatibilities

No pharmacologic incompatibilities have been demonstrated with quadrivalent HPV (Types 6, 11, 16, 18) recombinant vaccine. Results from clinical studies indicate that quadrivalent HPV vaccine may be administered concomitantly (at a separate injection site) with hepatitis B vaccine (recombinant). The safety of quadrivalent HPV vaccine when administered concomitantly with hepatitis B vaccine (recombinant) was evaluated in a placebo-controlled study. There were no statistically significant higher rates in systemic or injection-site adverse experiences among subjects who received concomitant vaccination compared with those who received quadrivalent HPV vaccine or hepatitis B vaccine alone. Co-administration of quadrivalent HPV vaccine with other vaccines has not been studied.

In clinical studies, use of hormonal contraceptives or lack of use of hormonal contraceptives among study participants did not alter vaccine efficacy.

11.1.4 Stability and Storage

Quadrivalent HPV (Types 6, 11, 16, 18) recombinant vaccine should be stored refrigerated at 2 to 8° C (36 to 46° F). The vaccine shelf life is 36 months from the date of manufacture when stored at 2 to 8° C. Vaccine should *not* be frozen and *should* be protected from light.

11.1.5 Toxicity Studies

Quadrivalent HPV (types 6, 11, 16, 18) recombiant vaccine has not been evaluated for the potential to cause carcinogenicity or genotoxicity. Quadrivalent HPV vaccine administration to female rats at a dose of 120 mcg total protein, which corresponds to approximately 300-fold excess relative to the projected human dose, had no effects on mating performance, fertility or embroyonic/fetal survival. Although no harm to the fetus due quadrivalent HPV vaccine was observed in pre-clinical animal studies, it is not known whether the vaccine can cause fetal harm when administered to a pregnant woman or if it can affect reproductive capacity. *Pregnancy is an exclusion criteria for participation in this protocol and a negative pregnancy test must be documented prior to administration of each dose of vaccine.*

An evaluation of the effect of quadrivalent HPV vaccine on embryo-fetal, pre- and postweaning development was conducted using rats. One group of rats was administered vaccine twice prior to gestation, during the period of organogenesis (gestation day 6) and on lactation day 7. A second group of pregnant rats was administered quadrivalent HPV vaccine during the period of organogenesis (gestation day 6) and on lactation day 7 only. Vaccine was administered at 0.5 mL/rat/occasion (approximately 300-fold excess relative to the projected human dose on a mg/kg basis) by intramuscular injection. No adverse effects on mating, fertility, pregnancy, parturition, lactation, embryo-fetal or pre- and postweaning development were observed. There were no vaccine-related fetal malformations or other evidence of teratogenesis noted in this study. In addition, there were no treatment-related effects on developmental signs, behavior, reproductive performance, or fertility of the offspring. The effect of quadrivalent HPV vaccine on male fertility has not been studied.

11.1.6 Vaccine-Associated Adverse Events

In study trials, the quadrivalaent HPV (types 6, 11, 16, 18) recombinant vaccine was well tolerated and few subjects (0.1%) discontinued due to adverse experiences. The vaccine-related adverse experiences that were observed among female vaccine recipients at a frequency of at least 1.0% and also at a greater frequency than that observed among placebo recipients included injection site pain, swelling, erythema and pruritus 1 to 5 days post-vaccination and fever 1 to 15 days post-vaccination. Overall, 94.3% of subjects who received the vaccine judged their injection-site reactions to be mild or moderate in intensity.

A total of 102 subjects out of 21,464 total subjects (9- to 26-year-old girls and women and 9- to 15-year-old boys) who received both quadrivalent HPV vaccine and placebo reported a serious adverse experience on Day 1-15 following any vaccination visit. The most frequently reported serious adverse experiences for the HPV vaccine compared to placebo regardless of causality were headache, gastroenteritis, appendicitis and pelvic inflammatory disease (all less than 0.1%). Across clinical studies, 17 deaths were reported in the 21,464 male and female subjects and were consistent with events expected in healthy adolescent and adult populations: motor vehicle accident (7), overdose/suicide (3) and pulmonary embolus/deep vein thrombosis (2). In addition, there were 2 cases of sepsis, 1 case of pancreatic cancer, and 1 case of arrhythmia in the HPV vaccine group and 1 case of asphyxia in the placebo group.

11.1.7 Vaccine Accountability

The entire supply of quadrivalent HPV (Types 6, 11, 16, 18) recombinant Vaccine to be administered through this study will be shipped to Judith M. Starling, Ph.D., of the Pharmaceutical Development Section, Department of Pharmacy, CC, NIH: Bldg. 10, Rm. 1D35 10 Center Drive MSC1196 Bethesda, MD 20892-1196. 301-496-1031. Upon dispensing vaccine, an appropriate procedure will be taken to ensure the accountability of each pre-filled syringe and its record will be attached to the appropriate Drug Administration page of the case report form to become part of the subject's permanent medical record. The person dispensing the product will initial and date the Drug Accountability Log as well as the dose dispensed. The label will contain the following information: subject identification number i.e. CC medical record number, protocol number, and the name of the study subject.

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13 STUDY APPENDICES

Appendix 1 Quadrivalent Human Papillomavirus Vaccine. Recommendations of the Advisory Committee on Immunization Practices. MMWR, March 12, 2007, 56:1-24.

Appendix 2 Schedule of Study Clinical and Laboratory Evaluations and Blood Volumes, HIV Seropositive Subjects (Cohorts 1 and 2)

Appendix 3 Schedule of Study Clinical and Laboratory Evaluations and Blood Volumes, HIV Seronegative Subjects (Cohort 3)

Appendix 4A HPV Vaccination Report Card Surveillance for Adverse Vaccine Experiences

Appendix 4B Instructions for Completing the HPV Vaccination Report

Appendix 5 Specifications for the Collection and Processing of HPV DNA Samples

Appendix 6 Specifications for the Collection, Processing and Shipping of Research Bloods

Appendix 7 Summary of Middle School and High School Risk Behavior Survey Questions

Appendix 8A Web Survey Information Sheet for Parents/Guardians of Protocol Participants

Appendix 8B Web Survey Information Sheet for Protocol Participants

Appendix 9 Middle School Risk Behavior Survey

Appendix 10 High School Risk Behavior Survey

Appendix 11 Protocol Summary and Overview for Informational and Recruitment Purposes

Appendix 1
Quadrivalent Human Papillomavirus Vaccine.
Recommendations of the Advisory Committee on Immunization Practices.

Date of ACIP vote: June 29, 2006
Date of posting of provisional recommendations: August 14, 2006
Date of publication of recommendations in CDC *Morbidity and Mortality Weekly Report*: March 12, 2007, 56:1-24.

Recommendations for use of quadrivalent HPV vaccine:

- Routine vaccination with three doses of quadrivalent HPV vaccine is recommended for females 11-12 years of age. The vaccination series can be started in females as young as 9 years of age.
- Catch-up vaccination is recommended for females 13-26 years of age who have not been vaccinated previously or who have not completed the full vaccine series. Ideally, vaccine should be administered before potential exposure to HPV through sexual contact.
- Each dose of quadrivalent HPV vaccine is 0.5 mL, administered intramuscularly.
- Quadrivalent HPV vaccine is administered in a three dose schedule. The second and third doses should be administered 2 and 6 months after the first dose.
- Quadrivalent HPV vaccine can be administered at the same visit when other age appropriate vaccines are provided, such as Tdap, Td and MCV4.
- At present, cervical cancer screening recommendations have not changed for females who receive quadrivalent HPV vaccine.

Special situations:

- Quadrivalent HPV vaccine can be given to females who have an equivocal or abnormal Pap test, a positive Hybrid Capture II® high risk test, or genital warts.

  Vaccine recipients should be advised that data from clinical trials do not indicate the vaccine will have any therapeutic effect on existing Pap test abnormalities, HPV infection or genital warts. Vaccination of these females would provide protection against infection with vaccine HPV types not already acquired.

- Lactating women can receive quadrivalent HPV vaccine

- Females who are immunocompromised either from disease or medication can receive quadrivalent HPV vaccine. However the immune response to vaccination and vaccine effectiveness might be less than in females who are immunocompetent.

Pregnancy:

- Quadrivalent HPV vaccine is not recommended for use in pregnancy.

  The vaccine has not been associated causally with adverse outcomes of pregnancy or adverse events to the developing fetus. However, data on vaccination during pregnancy are limited. Any exposure to vaccine during pregnancy should be reported to the vaccine pregnancy registry (1-800-986-8999).

Contraindications to use of vaccine:

- Quadrivalent HPV vaccine is contraindicated for people with a history of immediate hypersensitivity to yeast or to any vaccine component.

Precautions:

- Quadrivalent HPV vaccine can be administered to females with minor acute illnesses (e.g., diarrhea or mild upper respiratory tract infections, with or without fever). Vaccination of people with moderate or severe acute illnesses should be deferred until after the illness improves.

Appendix 2A

Schedule of Study Clinical and Laboratory Evaluations
HIV Seropositive Subjects (Cohorts 1 and 2)

	Screening D-14 to D-1	**Mo 0 D1**	**Mo 1**	**Mo 2**	**Mo 3**	**Mo 6**	**Mo 7**	**Mo 12**
Written Informed Consent	**X**							
Knowledge & Risk Behavior Survey		**X**						**X**
Medical History & Review of Systems	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Physical Examination	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Height	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Weight	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Vital Signs	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Chest X-Ray	**X**							
EKG	**X**							
Gynecologic Exam/Pap Smear/HPV DNA (Sexually Active Females Only)	**X**							**X**
Quadrivalent HPV Vaccine		**X**		**X**		**X**		
VRC Surveillance Report Card to Patient		**X**		**X**		**X**		
Clinical Laboratory Tests:								
CBC with differential	**X**		**X**	**X**	**X**	**X**	**X**	**X**
PT / PTT	**X**							
Acute Panel	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Hepatic Panel	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Mineral Panel	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Lipid Panel	**X**							**X**
Amylase / Lipase	**X**							**X**
Urinalysis	**X**							**X**
Serum Pregnancy Test	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Quantitative Igs & IgG Subclasses	**X**							**X**
HIV Serology	**X**							
Hepatitis B Serology (@ M7 if vaccinated)	**X**						**X**	
Hepatitis C Serology	**X**							
VDRL	**X**							
CD4 Cell Count (% and Absolute)	**X**		**X**	**X**	**X**	**X**	**X**	**X**
HIV-1 RNA level	**X**		**X**	**X**	**X**	**X**	**X**	**X**
HPV DNA Sampling:								
Buccal CytoBrush in PreservCyte (10ml)		**X**						
Males: Cytobrush® External Genitalia Cytobrush® Anogenital Verge		**X** **X**						
Females: Cytobrush® External Genitalia Cytobrush® Anogenital Verge		**X** **X**						
Merck HPV Antibody Assay		**X**					**X**	**X**
Research Studies:								
To Ligia Pinto, NCI FCRF: Functional HPV Antibody Assay Cryopreserved PBMCs for: HPV/HIV LPA, Cytokine Profiling		**X** **X**	**X** **X**		**X** **X**		**X** **X**	**X** **X**
Baselar Lab NCI FCRF: Extended FACS		**X**	**X**		**X**		**X**	**X**
Serum for Storage		**X**	**X**	**X**	**X**	**X**	**X**	**X**
Alter/Zheng Lab: Cryopreserved Whole Blood		**X**					**X**	**X**
Berzofsky Lab: Research Assays Immunologic Responses		**X**		**X**		**X**		

Appendix 2B

Schedule of Study Clinical and Laboratory Evaluations HIV Seropositive Subjects (Cohorts 1 and 2)

	Schedule of Study Events After Year 1					
	Mo 18	**Mo 24**	**Mo 30**	**Mo 36**	**Mo 42**	**Mo 48**
Medical History & Review of Systems	**X**	**X**	**X**	**X**	**X**	**X**
Physical Examination	**X**	**X**	**X**	**X**	**X**	**X**
Height	**X**	**X**	**X**	**X**	**X**	**X**
Weight	**X**	**X**	**X**	**X**	**X**	**X**
Vital Signs	**X**	**X**	**X**	**X**	**X**	**X**
Gynecologic Exam/Pap Smear/HPV DNA (Sexually Active Females Only)		**X**		**X**		**X**
Clinical Laboratory Tests:						
CBC with differential	**X**	**X**	**X**	**X**	**X**	**X**
Acute Panel	**X**	**X**	**X**	**X**	**X**	**X**
Hepatic Panel	**X**	**X**	**X**	**X**	**X**	**X**
Mineral Panel	**X**	**X**	**X**	**X**	**X**	**X**
Lipid Panel		**X**		**X**		**X**
Amylase / Lipase		**X**		**X**		**X**
Urinalysis		**X**		**X**		**X**
Serum Pregnancy Test	**X**	**X**	**X**	**X**	**X**	**X**
Quantitative Igs & IgG Subclasses		**X**		**X**		**X**
CD4 Cell Count (% and Absolute)	**X**	**X**	**X**	**X**	**X**	**X**
HIV-1 RNA level	**X**	**X**	**X**	**X**	**X**	**X**
Merck HPV Antibody Assay		**X**				**X**
Research Studies:						
To Ligia Pinto, NCI FCRF: Functional HPV Antibody Assay Cryopreserved PBMCs for: HPV/HIV LPA, Cytokine Profiling		**X** **X**		**X** **X**		**X** **X**
Baselar Lab NCI FCRF: Extended FACS	**X**	**X**	**X**	**X**	**X**	**X**
Serum for Storage	**X**	**X**	**X**	**X**	**X**	**X**
Alter/Zheng Lab: Cryopreserved Whole Blood		**X**		**X**		**X**

Appendix 2C

Blood Volumes for Clinical and Laboratory Evaluations HIV Seropositive Subjects (Cohorts 1 and 2)

	Screening D-14 to D-1	**Mo 0 D1**	**Mo 1**	**Mo 2**	**Mo 3**	**Mo 6**	**Mo 7**	**Mo 12**
Clinical Laboratory Tests:								
Light Lavender Tube K_2EDTA 3.0ml	**3.0 ml**		**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	
CBC with differential	X		X	X	X	X	X	X
Dark Lavender Tube K_2EDTA 6.0ml	**6.0 ml**		**6.0**	**6.0**	**6.0**	**6.0**	**6.0**	**6.0**
HIV-1 RNA level	X		X	X	X	X	X	X
CD4 Cell Count (% and Absolute)	X		X	X	X	X	X	X
Light Blue Tube Buffered Cit Na 2.7ml	**3.0 ml**							
PT / PTT	X							
Red/Yellow Rim SST Tube 4.0ml	**4.0 ml**		**4.0**	**4.0**	**4.0**	**4.0**	**4.0**	**4.0**
Acute Panel	X		X	X	X	X	X	X
Hepatic Panel	X		X	X	X	X	X	X
Mineral Panel	X		X	X	X	X	X	X
Lipid Panel	X							X
Amylase / Lipase	X							X
Serum Pregnancy Test	X		X	X	X	X	X	X
Quantitative Igs & IgG Subclasses	X							X
RPR	X							
Red/Yellow Rim SST Tube 8.0ml	**8.0 ml**							
Hepatitis B Serology	X							
Hepatitis C Serology	X							
Anti-HIV-1/2	X							
Red Plastic Clot Activator Tube 10.0ml		**10.0**					**10.0**	**10.0**
Merck HPV Antibody Assay		X					X	X
Research Studies:								
Green Stopper Na Heparin 10.0 ml 6 tubes		**60.0**	**60.0**		**60.0**		**60.0**	**60.0**
To Ligia Pinto, NCI FCRF: Functional HPV Antibody Assay Cryopreserved PBMCs for: HPV/HIV LPA, Cytokine Profiling		 X X	 X X		 X X		 X X	 X X
Green Stopper Na Heparin 10.0 ml 1tube		**10.0**	**10.0**		**10.0**		**10.0**	**10.0**
Baselar Lab NCI FCRF: Extended FACS		X	X		X		X	X
Red Plastic Clot Activator Tube 10.0 ml		**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**
Serum for Storage		X	X	X	X	X	X	X
Dark Lavender Tube 6.0 ml 3 Tubes		**18.0**					**18.0**	**18.0**
Alter/Zheng Lab: Cryopreserved Whole Blood		 X					 X	 X
Green Stopper Na Heparin 10.0 ml 2tubes		**20.0**						
Berzofsky Lab: Research Assays Immunologic Responses		 X						
Total Blood Volume in ml	**24.0 ml**	**128.0**	**93.0**	**23.0**	**93.0**	**23.0**	**121.0**	**121.0**
Total Blood Volume in ml per 6 weeks	**116.0**	**144.0**	**121.0**					

Appendix 2D

Blood Volumes for Clinical and Laboratory Evaluations HIV Seropositive Subjects (Cohorts 1 and 2)

	Schedule of Study Events After Year 1					
	Mo 18	**Mo 24**	**Mo 30**	**Mo 36**	**Mo 42**	**Mo 48**
Clinical Laboratory Tests:						
Light Lavender Tube K_2EDTA 3.0ml	**3.0 ml**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**
CBC with differential	X	X	X	X	X	X
Dark Lavender Tube K_2EDTA 6.0ml	**6.0 ml**	**6.0**	**6.0**	**6.0**	**6.0**	**6.0**
HIV-1 RNA level	X	X	X	X	X	X
CD4 Cell Count (% and Absolute)	X	X	X	X	X	X
Red/Yellow Rim SST Tube 4.0ml	**4.0 ml**	**4.0**	**4.0**	**4.0**	**4.0**	**4.0**
Acute Panel	X	X	X	X	X	X
Hepatic Panel	X	X	X	X	X	X
Mineral Panel	X	X	X	X	X	X
Lipid Panel		X		X		X
Amylase / Lipase		X		X		X
Serum Pregnancy Test	X	X	X	X	X	X
Quantitative Igs & IgG Subclasses		X		X		X
Red Plastic Clot Activator Tube 10.0ml		**10.0**				**10.0**
Merck HPV Antibody Assay		X				X
Research Studies:						
Green Stopper Na Heparin 10.0 ml 6 tubes		**60.0**		**60.0**		**60.0**
To Ligia Pinto, NCI FCRF: Functional HPV Antibody Assay Cryopreserved PBMCs for: HPV/HIV LPA, Cytokine Profiling		 X X		 X X		 X X
Green Stopper Na Heparin 10.0 ml 1tube	**10.0 ml**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**
Baselar Lab NCI FCRF: Extended FACS	X	X	X	X	X	X
Red Plastic Clot Activator Tube 10.0 ml	**10.0 ml**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**
Serum for Storage	X	X	X	X	X	X
Dark Lavender Tube 6.0 ml 3 Tubes		**18.0**		**18.0**		**18.0**
Alter/Zheng Lab: Cryopreserved Whole Blood		 X		 X		 X
Total Blood Volume in ml	**33.0**	**121.0**	**33.0**	**111.0**	**33.0**	**121.0**
Total Blood Volume in ml per 6 weeks	**33.0**	**121.0**	**33.0**	**111.0**	**33.0**	**121.0**

Appendix 3A

Schedule of Study Clinical and Laboratory Evaluations
HIV-*Negative* Control Subjects (Cohort 3)

	Screening D-14 to D-1	**Mo 0 D1**	**Mo 1**	**Mo 2**	**Mo 3**	**Mo 6**	**Mo 7**	**Mo 12**
Written Informed Consent	**X**							
Knowledge & Risk Behavior Survey		**X**						**X**
Medical History & Review of Systems	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Physical Examination	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Height	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Weight	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Vital Signs	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Chest X-Ray	**X**							
EKG	**X**							
Gynecologic Exam/Pap Smear/HPV DNA (Sexually Active Females Only)	**X**							**X**
Quadrivalent HPV Vaccine		**X**		**X**		**X**		
VRC Surveillance Report Card to Patient		**X**		**X**		**X**		
Clinical Laboratory Tests:								
CBC with differential	**X**		**X**	**X**	**X**	**X**	**X**	**X**
PT / PTT	**X**							
Acute Panel	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Hepatic Panel	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Mineral Panel	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Lipid Panel	**X**							**X**
Amylase / Lipase	**X**							**X**
Urinalysis	**X**							**X**
Serum Pregnancy Test	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Quantitative Igs & IgG Subclasses	**X**							**X**
HIV Serology	**X**							
Hepatitis B Serology (@ M7 if vaccinated)	**X**						**X**	
Hepatitis C Serology	**X**							
RPR	**X**							
CD4 Cell Count (% and Absolute)	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
HPV DNA Sampling:								
Buccal Brush in PreservCyte (10ml)		**X**						
Males: Cytobrush® External Genitalia Cytobrush® Anogenital Verge		**X** **X**						
Females: Cytobrush® External Genitalia Cytobrush® Anogenital Verge		**X** **X**						
Merck HPV Antibody Assay		**X**					**X**	**X**
Research Studies:								
To Ligia Pinto, NCI FCRF: Functional HPV Antibody Assay Cryopreserved PBMCs for: HPV LPA, Cytokine Profiling		**X** **X**	**X** **X**		**X** **X**		**X** **X**	**X** **X**
Serum for Storage		**X**	**X**	**X**	**X**	**X**	**X**	**X**
Alter/Zheng Lab: Cryopreserved Whole Blood		**X**					**X**	**X**

Appendix 3B

Schedule of Study Clinical and Laboratory Evaluations HIV-*Negative* Control Subjects (Cohort 3)

	Schedule of Study Events After Year 1					
	Mo 18	**Mo 24**	**Mo 30**	**Mo 36**	**Mo 42**	**Mo 48**
Medical History & Review of Systems	**X**	**X**	**X**	**X**	**X**	**X**
Physical Examination	**X**	**X**	**X**	**X**	**X**	**X**
Height	**X**	**X**	**X**	**X**	**X**	**X**
Weight	**X**	**X**	**X**	**X**	**X**	**X**
Vital Signs	**X**	**X**	**X**	**X**	**X**	**X**
Gynecologic Exam/Pap Smear/HPV DNA (Sexually Active Females Only)		**X**		**X**		**X**
Clinical Laboratory Tests:						
CBC with differential	**X**	**X**	**X**	**X**	**X**	**X**
Acute Panel	**X**	**X**	**X**	**X**	**X**	**X**
Hepatic Panel	**X**	**X**	**X**	**X**	**X**	**X**
Mineral Panel	**X**	**X**	**X**	**X**	**X**	**X**
Lipid Panel		**X**		**X**		**X**
Amylase / Lipase		**X**		**X**		**X**
Urinalysis		**X**		**X**		**X**
Serum Pregnancy Test	**X**	**X**	**X**	**X**	**X**	**X**
Quantitative Igs & IgG Subclasses		**X**		**X**		**X**
Merck HPV Antibody Assay		**X**				**X**
Research Studies:						
To Ligia Pinto, NCI FCRF:						
Functional HPV Antibody Assay		**X**		**X**		**X**
Cryopreserved PBMCs for:						
HPV LPA, Cytokine Profiling		**X**		**X**		**X**
Serum for Storage	**X**	**X**	**X**	**X**	**X**	**X**
Alter/Zheng Lab:						
Cryopreserved Whole Blood		**X**		**X**		**X**

Appendix 3C

Blood Volumes for Clinical and Laboratory Evaluations
HIV-*Negative* Control Subjects (Cohort 3)

	Screening D-14 to D-1	**Mo 0 D1**	**Mo 1**	**Mo 2**	**Mo 3**	**Mo 6**	**Mo 7**	**Mo 12**
Clinical Laboratory Tests:								
Light Lavender Tube K_2EDTA 3.0ml	**3.0 ml**		**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**
CBC with differential	X		X	X	X	X	X	X
Dark Lavender Tube K_2EDTA 6.0ml	**6.0 ml**		**6.0**	**6.0**	**6.0**	**6.0**	**6.0**	**6.0**
CD4 Cell Count (% and Absolute)	X		X	X	X	X	X	X
Light Blue Tube Buffered Cit Na 2.7ml	**3.0 ml**							
PT / PTT	**X**							
Red/Yellow Rim SST Tube 4.0ml	**4.0 ml**		**4.0**	**4.0**	**4.0**	**4.0**	**4.0**	**4.0**
Acute Panel	X		X	X	X	X	X	X
Hepatic Panel	X		X	X	X	X	X	X
Mineral Panel	X		X	X	X	X	X	X
Lipid Panel	X							X
Amylase / Lipase	X							X
Serum Pregnancy Test	X		X	X	X	X	X	X
Quantitative Igs & IgG Subclasses	X							X
RPR	X							
Red/Yellow Rim SST Tube 8.0ml	**8.0 ml**							
Hepatitis B Serology	X							
Hepatitis C Serology	X							
AntiHIV-1/2	X							
Red Plastic Clot Activator Tube 10.0 ml		**10.0**					**10.0**	**10.0**
Merck HPV Antibody Assay		X					X	X
Research Studies:								
Green Stopper Na Heparin 10.0 ml 6 tubes		**60.0**	**60.0**		**60.0**		**60.0**	**60.0**
To Ligia Pinto, NCI FCRF: Functional HPV Antibody Assay Cryopreserved PBMCs for: HPV LPA, Cytokine Profiling		 X X	 X X		 X X		 X X	 X X
Red Plastic Clot Activator Tube 10.0 ml		**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**
Serum for Storage		X	X	X	X	X	X	X
Dark Lavender Tube 6.0 ml 3 Tubes		**18.0**					**18.0**	**18.0**
Alter/Zheng Lab: Cryopreserved Whole Blood		 X					 X	 X
Total Blood Volume in ml	**24.0**	**98.0**	**83.0**	**23.0**	**83.0**	**23.0**	**111.0**	**111.0**
Total Blood Volume in ml per 6 weeks	**106.0**	**134.0**	**111.0**					

Appendix 3D

Blood Volumes for Study Clinical and Laboratory Evaluations HIV-*Negative* Control Subjects (Cohort 3)

	Schedule of Study Events After Year 1					
	Mo 18	**Mo 24**	**Mo 30**	**Mo 36**	**Mo 42**	**Mo 48**
Clinical Laboratory Tests:						
Light Lavender Tube K_2EDTA 3.0ml	**3.0 ml**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**
CBC with differential	X	X	X	X	X	X
Red/Yellow Rim SST Tube 4.0ml	**4.0 ml**	**4.0**	**4,0**	**4,0**	**4,0**	**4,0**
Acute Panel	X	X	X	X	X	X
Hepatic Panel	X	X	X	X	X	X
Mineral Panel	X	X	X	X	X	X
Lipid Panel		X		X		X
Amylase / Lipase		X		X		X
Serum Pregnancy Test	X	X	X	X	X	X
Quantitative Igs & IgG Subclasses		X		X		X
Red Plastic Clot Activator Tube 10.0 ml		**10.0**				**10.0**
Merck HPV Antibody Assay		X				X
Research Studies:						
Green Stopper Na Heparin 10.0 ml 6 tubes		**60.0**		**60.0**		**60.0**
To Ligia Pinto, NCI FCRF: Functional HPV Antibody Assay Cryopreserved PBMCs for: HPV LPA, Cytokine Profiling		X X		X X		X X
Red Plastic Clot Activator Tube 10.0 ml	**10.0 ml**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**
Serum for Storage	X	X	X	X	X	X
Dark Lavender Tube 6.0 ml 3 Tubes		**18.0**		**18.0**		**18.0**
Alter/Zheng Lab: Cryopreserved Whole Blood		 X		 X		 X
Total Blood Volume in ml	**17.0**	**105.0**	**17.0**	**95.0**	**17.0**	**95.0**
Total Blood Volume in ml per 6 weeks	**17.0**	**105.0**	**17.0**	**95.0**	**17.0**	**95.0**

Appendix 4A

NCI Vaccine Branch HPV Vaccination Report Card

Protocol Number _____ **Date** _____

Patient Name _____ **MR** _____

Research Nurse Contact information: Claudia Derse-Anthony 301-443-4237 / derseanthonycp@mail.nih.gov or Brenda Roberson 301.435.4733 / broberson@mail.nih.gov

THIS VACCINATION REPORT CARD IS VERY IMPORTANT TO THIS STUDY
PLEASE RETURN COMPLETED REPORT CARD 15 DAYS AFTER YOUR VACCINE INJECTION USING THE RETURN ENVELOPE ENCLOSED.

RETURN DATE: _____

RECORD YOUR INJECTION SITE REACTIONS EVERY DAY FOR 5 DAYS

Injection site: ☐ Right Arm (RA) ☐ Left Arm (LA) ☐ Other _____
☐ Right Thigh (RT) ☐ Left Thigh (LT)

	DAY 1 **Vaccination Day** _____ month/day/year (4 hours after injection)	DAY 2 _____ month/day/year	DAY 3 _____ month/day/year	DAY 4 _____ month/day/year	DAY 5 _____ month/day/year	**LAST DATE REACTION PRESENT**
SWELLING	☐ None ☐ 1 ☐ 2 ☐ 3 ☐ over3:__	☐ None ☐ 1 ☐ 2 ☐ 3 ☐ over3:__	☐ None ☐ 1 ☐ 2 ☐ 3 ☐ over3:__	☐ None ☐ 1 ☐ 2 ☐ 3 ☐ over3:__	☐ None ☐ 1 ☐ 2 ☐ 3 ☐ over3:__	
REDNESS	☐ None ☐ 1 ☐ 2 ☐ 3 ☐ over3:__	☐ None ☐ 1 ☐ 2 ☐ 3 ☐ over3:__	☐ None ☐ 1 ☐ 2 ☐ 3 ☐ over3:__	☐ None ☐ 1 ☐ 2 ☐ 3 ☐ over3:__	☐ None ☐ 1 ☐ 2 ☐ 3 ☐ over3:__	
PAIN or *Tenderness*	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	
Other Injection Site Reaction (describe)	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	

NCI Vaccine Branch
HPV Vaccination Report Card

Protocol Number _____ **Date** _____
Patient Name _____ **MR** _____
Research Nurse Contact information: Brenda Roberson 301.435.4733 / broberson@mail.nih.gov

RECORD YOUR TEMPERATURE *EVERY DAY* FOR 5 DAYS

DAY	DATE/TIME	ORAL TEMPERATURE
1		
2		
3		
4		
5		

RECORD OTHER SYMPTOMS ASSOCIATED WITH YOUR VACCINATION

	DAY 1 Vaccination Day _____ month/day/year (4 hours after injection)	DAY 2 _____ month/day/year	DAY 3 _____ month/day/year	DAY 4 _____ month/day/year	DAY 5 _____ month/day/year	**LAST DATE REACTION PRESENT**
Other Symptoms (describe)	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	
Other Symptoms (describe)	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	

RECORD SYMPTOMS THAT BEGIN 6 OR MORE DAYS FOLLOWING YOUR VACCINATION (include severity of symptom, start date and the last day it was present).

Injection Site Reactions or Other Symptoms Beginning 6 or More Days After Vaccination	**DATE (month/day/year)**		SEVERITY
	Started	**Last Present**	
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe

_____ _____
Name of Person Completing Vaccine Report Card **Date**

Appendix 4B

Instructions for Completing Your HPV Vaccination Report Card

Research Nurse Contact information: Brenda Roberson 301.435.4733 / broberson@mail.nih.gov

What vaccination reactions can you expect?

Some patients who receive IM (into the muscle) vaccinations may experience some swelling and redness at the injection site. There may also be pain or muscle soreness for 1 to 2 days following the vaccine injection. Rarely patients may develop a blister type sore at the site with an area of redness surrounding the blister while others may not have any of these reactions. Some patients also experience generalized reactions such as feeling tired and achy (commonly called malaise or fatigue) or possible headache and fever. These symptoms are temporary and can be relieved with acetaminophen ("Tylenol") or ibuprofen ("Motrin"). Whatever symptoms you have related to your vaccine injection, it is important that you record <u>all of them</u> on your Vaccine Report card.

Instructions for measuring injection site reactions:

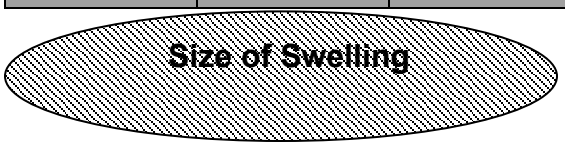
Please measure the size of any swelling or redness at the injection site using the ruler marker at the bottom of the page. Measure from edge to edge and document the size on your report card. You may have any one reaction or a combination of reactions.

Directions for measuring the size of the injection reaction:

1	2	3	4	5	6

This reaction measures 2 inches at its largest width so you would check the box marked "2" under **REDNESS** on the <u>Injection site reaction</u> section of the report card.

1	2	3	4	5	6



This reaction measures 3 at its largest width so you would check the box marked "3" under SWELLING on the <u>Injection site reaction</u> section of the report card.

Ruler marker

1	2	3	4	5	6

Appendix 4B

Instructions for Completing Your HPV Vaccination Report Card

Directions for Documenting Injection Site Reactions:

Pain, Tenderness, or Other Reactions

Mark the box that best describes the severity of the injection site reaction using the following definitions:

- **None** is without a reaction or symptom
- **Mild** is awareness of symptom, but easily tolerated
- **Moderate** is discomfort enough to cause interference with usual activates
- **Severe** is incapacitation with inability to work or do usual activity

Other injection site reactions may include symptoms of itching or burning, blisters or ulcerations. If you have blisters, ulcerations, any type of skin breakdown or if you are concerned at all about infection at your injections site, contact the study research nurse Brenda Roberson or Dr. Wood immediately.

Temperature Measurement

- It is important that you take your temperature close to the same time every day for 5 days starting with the day of the vaccination.
- Take your temperature in the evening whenever possible. If you need to take your temperature more than once during a day, record the highest temperature taken that day.
- Take your temperature orally and record this temperature in the appropriate box.

Recording Other Symptoms Associated with Your Vaccination

If you have any generalized symptoms, such as feeling tired or achy, if you have a fever, headache or any other symptoms, record them in this section. Mark the box that best describes the severity of your symptoms using the following definitions:

- **None** is without a reaction or symptom
- **Mild** is awareness of symptom, but easily tolerated
- **Moderate** is discomfort enough to cause interference with usual activates
- **Severe** is incapacitation with inability to work or do usual activity

Symptoms that Begin 6 or More Days Following Your Vaccination

Sometimes individuals have delayed reactions to vaccines that don't appear until several days following the vaccination. If you have any symptoms at the injection site or other generalized symptoms that you ***think might be related*** to the HPV vaccine, please record them. We are interested in determining whether there are any problems with delayed reactions to the vaccination i.e. from 6 days up to 15 days following the vaccination.

Please remember to *sign your name* and *date* your HPV Vaccination Report Card before returning it to Brenda Roberson, RN, the research study coordinator.

Appendix 5

Specifications for the Collection and Processing of HPV DNA Samples

At study month 0, oral/buccal and external anogenital sampling will be performed to obtain clinical specimens for detection and characterization of HPV DNA positivity. This sampling will be performed only one time at study entry, and will provide an opportunity to characterize HPV DNA findings in the study cohort populations in a descriptive manner. As part of the informed consent and assent process, the Principal Investigator or their designee and members of the clinical research team will provide a detailed overview of the procedures involved in sampling, including physical demonstration of Buccal Cytobrush in PreservCyt 10ml and external anogenital (Medscand Cytobrush® plus) sampling utilizing the respective collection devices specified. Parents or patients that are uncomfortable allowing sampling for HPV DNA in the described manner may decline to have the sampling performed. Declination of HPV DNA sampling will not affect the subject's participation in all other aspects of the protocol. In addition to oral/buccal and external anogenital sampling at study entry, *sexually active* females will undergo gynecologic evaluation, cervical cytology (Pap smear) and commercially available diagnostic HPV DNA testing at screening, month 12 and annually thereafter as outlined in sections 2.2.1, 3.5.2 and 3.5.3, respectively.

Type specific HPV DNA testing will be performed by Roche Molecular Diagnostics:

Contact: Carrie Aldrich, Research Leader
Phone: 925-730-8607
Email: carrie.aldrich@roche.com

HPV CT/NG Life Cycle Team
Roche Molecular Diagnostics
Department of Medical Affairs
4300 Hacienda Drive
Pleasanton, CA 94588

Appendix 6

Specifications for the Collection, Processing and Shipping of Research Bloods

Clinical samples will be obtained for basic investigation of humoral and cellular immune responses induced by quadrivalent HPV vaccination.

Serum for HPV antibody titers performed by Merck (competitive luminex assay)

- Study Subjects: All
- Collection Tube: 10ml RTT
- Assay Timepoints: 0, 1, 2, 3, 6, 7, 12, 18, 24, 30, 36, 42, 48
- Send via courier to NCI FCRF for processing, aliquoting and cryopreservation by Baselar lab.
- Batched specimens to be sent to Merck for HPV antibody titers.

Studies to be performed by Dr. Ligia Pinto, NCI, Frederick:
Functional HPV antibody neutralization assays

- Study Subjects: All
- Collection Tube: Aliquot to be used from serum storage
- Assay timepoints: 0, 1, 3, 7, 12, 24, 36, 48

Cryopreserved PBMCs for HPV & HIV lymphocyte proliferation assays, cytokine induction assay, luminex multi-cytokine profiling of PBMCs. Note: HIV lymphocyte proliferation assays will only be performed in HIV-infected subjects.

- Study Subjects: All
- Collection Tube: 6 tubes sodium heparin 10ml GTT (60ml total)
- Assay Timepoints: 0, 1, 3, 7, 12, 24, 36, 48
- Send via courier to NCI FCRF for processing, aliquoting and cryopreservation by Baselar lab according to protocol provided by L. Pinto.

Studies to be performed by Dr. Mike Baselar / Dr. Bill Kopp, NCI, Frederick:
Extended FACS/Quantitative lymphocyte subpopulation studies

- Study Subjects: HIV-infected subjects only (Cohorts 1 and 2)
- Collection tube: 10ml sodium heparin GTT
- Assay Timepoints: 0, 1, 3, 7, 12, 18, 24, 30, 36, 42, 48

Serum Storage (for L. Pinto functional HPV neutralization antibody assay and for possible later quantitative measurement of HIV-related chemokines MIP-1α, MIP-1β and RANTES)

- Study Subjects: All
- Collection Tube: 10ml RTT
- Assay Timepoints: 0, 1, 2, 3, 6, 7, 12, 18, 24, 30, 36, 42, 48
- Send via courier to NCI FCRF for processing, aliquoting and cryopreservation by Baselar lab.

Cryopreserved whole blood for HPV DNA testing, Drs. Harvey Alter & Thomas Zheng

- Study Subjects: All
- Collection Tube: 6ml purple top EDTA tubes; collect 3 tubes (18ml total)
- Assay Timepoints: 0, 7, 12, 24, 36, 48

Research assays immunologic responses, Dr. Jay Berzofsky

- Study Subjects: HIV infected subjects only (Cohorts 1 and 2)
- Collection Tube: 10ml sodium heparin GTT; collect 2 tubes (20ml total)
- Assay Timepoints: Month 0 only

Appendix 7

Summary of Middle School and High School Risk Behavior Survey Questions

Middle School Survey Grades 6-8, Ages 11 – 13 yrs Total Estimated Survey Time: 15 – 30 minutes			**High School Survey Grades 9-12 and Graduates, Ages ≥ 14 yrs Total Estimated Survey Time: 15- 30 minutes**		
Survey Section	**No. Questions**	**Question Sequence**	**Survey Section**	**No. Questions**	**Question Sequence**
A. Demographics	**7**	**A1 – A7**	**A. Demographics**	**7**	**A1 – A7**
B. HPV/HIV Knowledge	**37**	**B1 – B37**	**B. HPV/HIV Knowledge**	**37**	**B1 – B37**
HPV Knowledge	11	B1 – B11	HPV Knowledge	11	B1 – B11
HIV Knowledge	26	B12 – B37	HIV Knowledge	26	B12 – B37
C. Risk Behaviors (YRBSS)	**26**	**C1 – C29**	**C. Risk Behaviors (YRBSS)**	**71**	**C1 – C57**
Safety	4	C1 – C4	Safety	4	C1 – C4
Violence	3	C5 – C7	Violence	11	C5 – C15
Tobacco Use	8	C8 – C15	Tobacco Use	11	C16 – C26
Alcohol Use	2	C16 – C17	Alcohol Use	6	C27 - C32
Marijuana Use	2	C18 – C19	Marijuana Use	4	C33 – C36
Other Drug Use	3	C20 – C22	Other Drug Use	9	C37 – C45
Sexual Behaviors	4	C23 – C26	Sexual Behaviors*	26	C46 – C71
Total # of Survey Questions:	**70**		**Total # of Survey Questions:**	**115**	
* High School Survey Sexual Behavior questions include CDC YRBS questions (C51 – C57) and additional questions about specific sexual practices (questions C58 – C76) developed by Dr. Lori Wiener.					

The Middle School and High School Surveys used in this clinical study have been adapted from the 2007 CDC National Youth Risk Behavior Surveys (YRBS) (refer to http://www.cdc.gov/healthyYouth/yrbs/questionnaire-txt.htm). YRBS are administered to a representative sample of middle and high school students who attend public and private schools across the United States as part of the Youth Risk Behavior Surveillance System (YRBSS) (refer to http://www.cdc.gov/HealthyYouth/yrbs/index.htm). The YRBSS monitors six categories of priority health-risk behaviors among youth and young adults, including behaviors that contribute to unintentional injuries and violence; tobacco use; alcohol and other drug use; sexual behaviors that contribute to unintended pregnancy and sexually transmitted diseases (STDs), including human immunodeficiency virus (HIV) infections; unhealthy dietary behaviors; and physical inactivity. The CDC developed the YRBSS to monitor priority health-risk behaviors among youth and young adults in each of these six categories and general health status, overweight and asthma. YRBSS includes national, state, and local school based surveys that have been conducted biennially since 1991.[1] Specifically, YRBS data are used to: measure progress toward achieving 15 national health objectives for Healthy People 2010 and three of the leading health indicators; to asses trends in priority health-risk

Appendix 7

Summary of Middle School and High School Risk Behavior Survey Questions

behaviors among students; and to evaluate the impact of broad school and community interventions at the national, state and local levels.[2]

The demographic section is identical in content to the CDC 2007 YRBS for both middle and high school with two additional question added: "Who do you live with?" and "Do you have HIV infection?". The first question is very relevant to the study cohort of HIV-infected patients to be enrolled, as many do not live in traditional nuclear family structures because of the loss of one or both parents to HIV disease. The second question was added so as to allow comparison of survey responses between HIV-positive and HIV-negative study participants.

Sections from the 2007 YRBS surveys that have been omitted from the surveys used in this protocol include questions regarding:

- Body weight (7 questions)
- Food (8 questions) (in High School Survey only)
- Physical activity (5 questions)
- Other health-related topics (3 questions)
- Suicide (3 questions Middle School Survey, 5 questions High School Survey)

Risk behavior questions on both surveys are identical in section content, sequence and total number of questions to the 2007 YRBS. The HIV knowledge assessment component of the surveys was developed by the Principal Investigator and Lori Wiener, Ph.D. on which they have published.[3] HPV knowledge questions in the survey have not been formally evaluated for content as no survey instrument regarding HPV knowledge has been validated in the adolescent or young adult populations. Additional questions added to the High School Risk Behavior Survey about specific sexual practices (questions C58 – C76) were developed by Lori Wiener, Ph.D. based on surveys of additional sexual practices among adolescents conducted by the Kaiser Family Foundation.[4]

1. Berner ND, Kann L, Kinchen S, et al. Methodology of the youth Risk Behavior Surveillance System. *MMWR* 2004;53(RR-12):1-13.
2. Eaton DK, Kann L, Kinchen S et al. Youth risk behavior surveillance – United States, 2002. *MMWR* 2006:55(SS-5):1-112.
3. Wiener LS, Battles HB, Wood LV. A longitudinal study of adolescents with perinatally or transfusion acquired HIV infection: sexual knowledge, risk reduction self-efficacy and sexual behavior. AIDS Behav. 2007 May;11(3):471-8
4. Henry J. Kaiser Foundation, Hoff T, Greene L and Davis J. National survey of adolescents and young adults: sexual health knowledge, attitudes and experiences. ©2003 Henry J. Kaiser Family Foundation, Menlo Park, California.

Appendix 8A

Web Survey Information Sheet for Parents/Guardians of Protocol Participants

As part of the protocol your child/teen is participating in, we are conducting a survey of behaviors that may affect their health. The responses to this survey will help us develop better age-appropriate health education information.

The survey asks about behaviors and behavior choices that young people are commonly faced with. Specifically it asks about activities that potentially put individuals at risk for HIV, HPV, and other sexually transmitted diseases. It also contains questions that help us gauge your child or teen's basic understanding and knowledge about HIV and HPV. The survey questions are different for middle school and high school aged participants. As a parent/legal guardian these surveys are available for you to read and review.

The survey is being conducted online to protect your child/teen's privacy and to ensure that the results are completely confidential. This confidentiality encourages participants to answer questions based on what they really do and not on what people expect them to say. The answers given will be kept private and cannot be traced back to a specific individual. Only investigators associated with the protocol will be able to access the data collected by the survey.

Important things for you as a parent/guardian to know about the survey:

1. Participation in this survey is *totally voluntary*. Whether or not you allow your child/teen to participate will not affect their participation in the HPV vaccine protocol. They will still receive the HPV vaccine and be monitored on study.
2. The survey is taken over the web to protect your child/teen's confidentiality.
3. When your child/teen accesses the web survey site, the first thing they will be asked to do is to create an alias. An alias is a name that is made up that no one else will know. For example if your name is Jane Doe, you could put Daffodil as your alias, if that happened to be your favorite flower. Your child/teen's alias can be any name they choose.
4. The questions that ask about your child/teen's background (including their date of birth) and HIV status are used only to describe the participants completing the survey. The information will not be used to find out their name. No names will ever be reported.
5. The survey requires that an answer be provided to every question in order to go to the next page.
6. For some questions, if a certain answer is chosen other questions will automatically be omitted.
7. It should take your child/teen no more than 20 to 30 minutes to complete this survey.

To familiarize you with what it is like to complete a web survey online through Survey Monkey and replicate the survey format your child/teen will experience, we have developed a brief ten-question survey. If you choose to, the survey should take less than five minutes to complete.

You can access this survey at
https://www.surveymonkey.com/s.aspx?sm=meEqnACczVvAw4LZMnLD0A_3d_3d.
The password to access the survey is PARENT.

Thank you for considering your child/teen's participation in this survey. Feel free to contact Dr. Wood or Dr. Wiener if you have any additional questions.

Appendix 8B

Web Survey Information Sheet for Protocol Participants

As part of the protocol you are participating in, we are conducting a survey of behaviors that may affect your health. The responses to this survey will help us develop better health education information relevant to people your own age.

The survey asks about behaviors and behavior choices that young people are commonly faced with. Specifically it asks about activities that potentially put individuals at risk for HIV, HPV, and other sexually transmitted diseases. It also contains questions that help us gauge your basic understanding and knowledge about HIV and HPV.

We are conducting this survey online to protect your privacy and to ensure that the results are completely confidential. The answers you give will be kept private. No one will know what you answer. Answer the questions based on what you really do.

Important things for you to know about this survey:

1. Participation in this survey is *totally voluntary.* Whether or not you choose to participate will not affect your participation in the HPV vaccine protocol. You will still receive the HPV vaccine and be monitored on study.
2. The survey is taken over the web to protect your confidentiality.

   If you are in MIDDLE SCHOOL you can access this survey at https://www.surveymonkey.com/s.aspx?sm=8253zuHkZF5AOYVQ_2bstL9w_3d_3d The password to access the survey is MSHPV.

   If you are in HIGH SCHOOL you can access this survey at https://www.surveymonkey.com/s.aspx?sm=nOhjdt_2fySXHI_2fS_2fcGIQMjw_3d_3d The password to access the survey is HSHPV.

3. When you access the survey site, the first thing you will be asked to do is to create an alias. An alias is a name that you make up that no one else will know. For example if my name is Jane Doe, for my alias I could put Daffodil if that was my favorite flower. Your alias can be any name you want just be sure that you can easily remember it.
4. The questions that ask about your background (including your date of birth) and HIV status will be used only to describe the participants completing the survey. It is important that you enter your date of birth correctly so the database can calculate how old you are at the time you take this survey. The information will not be used to find out your name. No names will ever be reported.
5. Make sure to read every question. The survey requires that you provide an answer for every question in order to go to the next page.
6. For some questions, if you choose a certain answer other questions will automatically be omitted.
7. It should take only about 15 to 30 minutes to complete this survey.
8. To begin the survey, just click on the answer that you want and at the end of the page click next and you're on your way!

Thank you for taking the time to complete this survey. If you have any questions, please contact Dr. Wood or Dr. Wiener.

Appendix 9

Middle School Risk Behavior Survey

You are currently enrolled in study of a recently approved quadrivalent HPV vaccine. The brand name of this vaccine is GARDASIL®. It is the first vaccine approved for use in pre-teens, teenagers and young adults to prevent human papillomavirs or HPV. HPV is a virus that is transmitted by sex and is associated with development of cancers of the anus and genital tract in both males and females. This survey is about health behaviors and your knowledge about HPV and human immunodeficiency virus (HIV- the virus that causes AIDS). It has been developed so you can tell us what you know about HPV and HIV and what you do that may affect your health. The information you give will be used to help us better understand how to provide better health education for young people like yourself.

The questions in the survey about health behaviors are identical to questions on the CDC's Youth Risk Behavior Survey that is administered nationally every 2 years to students across the United States attending public and private middle schools and high schools. The investigators conducting this quadrivalent HPV vaccine study have added additional questions specific about HPV and HIV because we are giving the vaccine to teens and young adults who are HIV positive as well as those who are HIV negative.

This is a web-based survey that will be administered via the internet. Similar to when you log on to "My Space" or other websites that have restricted access, you will need to decide on a unique USER ID (an alias). A password code will be provided to you to access and complete the survey over the internet.

You will NOT be asked to give your name. However you will be asked for background information (including your date of birth) and HIV status. It is important that you enter your date of birth accurately so the database can correctly calculate how old you are at the time you take this survey.

Important Things You Should Know:

- The answers you give will be kept private. No one will know what answer you select to any of the questions.
- Answer the questions based on what you really do.
- Completing the survey is entirely voluntary. Whether or not you answer the questions will not affect your participation in this vaccine study. *If you are not comfortable answering a question, just leave it blank.*
- The questions that ask about your background will be used only to describe the people who are completing this survey. The information will not be used to find out your name. *No names will ever be reported.*
- Make sure to read every question and then select the one answer that best describes what you think or what you do.
- When you are finished, follow the instructions of the study staff that helped you log on to the web site.

Thank you very much for your help.

A. Background Information

A1. Are you: ☐ Male ☐ Female

A2. What is your date of birth? ___ / ___ / _____
dd mm yyyy

A3. Are you Hispanic or Latino?
☐ Yes ☐ No

A4. What is your race? ***[Check <u>all</u> that apply]***
☐ Black or African American ☐ White
☐ American Indian or Alaskan Native ☐ Asian
☐ Native Hawaiian/other Pacific Islander ☐ Other _____

A5. Are you currently in school?
☐ Yes ↓ ☐ No ↓

A5A. What grade are you in?
☐ Less than 6th grade
☐ 6th grade
☐ 7th grade
☐ 8th grade
☐ 9th grade
☐ 10th grade
☐ 11th grade
☐ 12th grade
☐ College or vocational school

A5B. What was the highest level of education you completed?
☐ Middle school
☐ Some high school
☐ High school or equivalent (GED)
☐ Some college/A.A. degree/vocational training
☐ College degree/postgraduate

A6. Who do you live with?
☐ I live by myself
☐ I live with my biologic parents
☐ I live with my foster parents
☐ I live with my adoptive parents
☐ I live with other relatives
☐ I live with a roommate(s)
☐ I live with a boyfriend, girlfriend, or spouse
☐ Other (Specify: _____)

A7. Do you have HIV infection? ☐ Yes ☐ No

B. The next 11 questions are about human papillomavirus or HPV.

B1. Have you heard of human papillomavirus, or HPV?

- ☐ Yes
- ☐ No ⟶ ***Skip to question 12***

B2. How did you hear about it?

- ☐ A doctor, nurse or other health care provider
- ☐ Friend or family member
- ☐ Internet
- ☐ TV
- ☐ Magazine or newspaper
- ☐ Other (specify: _____)

B3. HPV is sexually transmitted.

- ☐ True
- ☐ False
- ☐ Don't know

B4. HPV is the main cause of cervical cancer.

- ☐ True
- ☐ False
- ☐ Don't know

B5. Who can carry HPV?

- ☐ Men
- ☐ Women
- ☐ Men and Women
- ☐ Neither men nor women
- ☐ Don't know

B6. Genital warts cause cervical cancer.

- ☐ True
- ☐ False
- ☐ Don't know

B7. The pill protects against HPV.

- ☐ True
- ☐ False
- ☐ Don't know

B8. Condoms provide total protection against HPV.

- ☐ True
- ☐ False
- ☐ Don't know

B9. People can have HPV and not have any symptoms.

- ☐ True
- ☐ False
- ☐ Don't know

B10. A pap smear can tell you if you've been exposed to HPV.

- ☐ True
- ☐ False
- ☐ Don't know

B11. HPV can be transmitted by

- ☐ Oral sex
- ☐ Vaginal sex
- ☐ Anal sex
- ☐ Any genital contact (including fingers)
- ☐ All of the above
- ☐ None of the above
- ☐ Don't know

B. The next 26 questions are about human immunodeficiency virus or HIV.

B. Can HIV can be spread through:	**Yes**	**No**	**Don't know**
Blood	☐	☐	☐
B13. Vaginal fluids	☐	☐	☐
B14. Saliva	☐	☐	☐
B15. Semen	☐	☐	☐
B16. Breast milk	☐	☐	☐
B17. Skin contact	☐	☐	☐

	Can HIV be spread through any of the following behaviors?	Yes	No	Don't know
B18.	Kissing	☐	☐	☐
B19.	Oral sex	☐	☐	☐
B20.	Vaginal Sex	☐	☐	☐
B21.	Eating or drinking from common utensils	☐	☐	☐
B22.	Anal sex	☐	☐	☐
B23.	Living with an HIV-infected person	☐	☐	☐
B24.	Mosquitos	☐	☐	☐
B25.	Sharing needles with an HIV-infected person	☐	☐	☐
B26.	Needle stick injuries (a health care worker being stuck with an infected needle)	☐	☐	☐
B27.	Piercing or tattooing with non-sterile needles	☐	☐	☐
B28.	Sneezing or coughing	☐	☐	☐
B29.	Shaking hands	☐	☐	☐
B30.	Hugging	☐	☐	☐
B31.	Using the same restroom as an HIV-infected person	☐	☐	☐
B32.	Drinking from the same water fountain as an HIV-infected person	☐	☐	☐

	Can HIV can be transmitted by:	Yes	No	Don't know
B33.	An HIV-infected person with an undetectable level of virus	☐	☐	☐
B34.	An HIV-infected person with no symptoms	☐	☐	☐
B35.	An HIV-infected person who has not been diagnosed with AIDS	☐	☐	☐
B36.	An HIV-infected person who is taking medications	☐	☐	☐
B37.	An HIV-infected woman to her unborn child.	☐	☐	☐

C. The next 4 questions ask about personal safety.

C1. **When you ride a bicycle,** how often do you wear a helmet?

- ☐ I do not ride a bicycle
- ☐ Never wore a helmet
- ☐ Rarely wore a helmet
- ☐ Sometimes wore a helmet
- ☐ Most of the time wore a helmet
- ☐ Always wore a helmet

C2. **When you rollerblade or ride a skateboard,** how often do you wear a helmet?

- ☐ I do not rollerblade or ride a skateboard
- ☐ Never wore a helmet
- ☐ Rarely wore a helmet
- ☐ Sometimes wore a helmet
- ☐ Most of the time wore a helmet
- ☐ Always wore a helmet

C3. How often do you wear a seat belt when **riding in** a car?

- ☐ Never
- ☐ Rarely
- ☐ Sometimes
- ☐ Most of the time
- ☐ Always

C4. Have you ever ridden in a car driven by someone who had been drinking alcohol?

- ☐ Yes
- ☐ No
- ☐ Not sure

C. The next 3 questions ask about violence-related behaviors.

C5. Have you ever carried **a weapon** such as a gun, knife or club?

- ☐ Yes
- ☐ No

C6. Have you ever been in a physical fight?

- ☐ Yes
- ☐ No

C7. Have you ever been in a physical fight in which you were hurt and had to be treated by a doctor or nurse?

- ☐ Yes
- ☐ No

C. The next 8 questions ask about tobacco use.

C8. Have you ever tried cigarette smoking, even one or two puffs?

- ☐ Yes
- ☐ No

C9. How old were you when you smoked a whole cigarette for the first time?

- ☐ I have never smoked a whole cigarette
- ☐ 8 years old or younger
- ☐ 9 years old or
- ☐ 10 years old
- ☐ 11 years old
- ☐ 12 years old
- ☐ 13 years old or older

C10. During the past 30 days on how many days did you smoke cigarettes?

- ☐ 0 Days
- ☐ 1 or 2 days
- ☐ 3 to 5 days
- ☐ 6 to 9 days
- ☐ 10 to 19 days
- ☐ 20 to 29 days
- ☐ All 30 days

C11. During the past 30 days, on the days you smoked, how many cigarettes did you smoke **per day**?

- ☐ I did not smoke cigarettes during the past 30 days
- ☐ Less than 1 cigarette per day
- ☐ 1 cigarette per day
- ☐ 2 to 5 cigarettes per day
- ☐ 6 to 10 cigarettes per day
- ☐ 11 to 20 cigarettes per day
- ☐ More than 20 cigarettes per day

C12. During the past 30 days, how did you **usually** get your own cigarettes? Select only **one** response.

- ☐ I did not smoke cigarettes during the past 30 days
- ☐ I bought them in a store such as a convenience store, supermarket, discount store or gas station.

- ☐ 1 bought them from a vending machine.
- ☐ I gave someone else money to buy them for me.
- ☐ I borrowed (or bummed) them from someone else.
- ☐ A person 18 years old or older gave them to me.
- ☐ I took them from a store or family member.
- ☐ I got them some other way.

C13. Have you ever smoked cigarettes daily, that is, at least one cigarette every day for 30 days?

- ☐ Yes
- ☐ No

C14. During the past 30 days, on how many days did you use **chewing tobacco, snuff, or dip,** such as Redman, Levi Garrett, Beechnut, Skoal, Skoal Bandits, or Copenhagen?

- ☐ 0 days
- ☐ 1 or 2 days
- ☐ 3 to 5 days
- ☐ 6 to 9 days
- ☐ 10 to 19 days
- ☐ 20 to 29 days
- ☐ All 30 days

C15. During the past 30 days, on how many days did you smoke **cigars, cigarillos, or little cigars**?

- ☐ 0 days
- ☐ 1 or 2 days
- ☐ 3 to 5 days
- ☐ 6 to 9 days
- ☐ 10 to 19 days
- ☐ 20 to 29 days
- ☐ All 30 days

C. The next 2 questions ask about drinking alcohol. This includes drinking beer, wine, wine coolers, and liquor such as rum, gin, vodka, or whiskey. For these questions, drinking alcohol does not include drinking a few sips of wine for religious purposes.

C16. Have you ever had a drink of alcohol, other than a few sips?

- ☐ Yes
- ☐ No

C17. How old were you when you had your first drink of alcohol other than a few sips?

- ☐ I have never had a drink of alcohol other than a few sips
- ☐ 8 years old or younger
- ☐ 9 years old
- ☐ 10 years old
- ☐ 11 years old
- ☐ 12 years old
- ☐ 13 years old or older

C. The next 2 questions ask about marijuana use. Marijuana also is called grass or pot.

C18. Have you ever used marijuana?

- ☐ Yes
- ☐ No

C19. How old were you when you tried marijuana for the first time?

- ☐ I have never tried marijuana
- ☐ 8 years old or younger
- ☐ 9 years old
- ☐ 10 years old
- ☐ 11 years old
- ☐ 12 years old
- ☐ 13 years old or older

C. The next 3 questions ask about other drugs.

C20. Have you ever used **any** form of cocaine, including powder, crack, or freebase?

- ☐ Yes
- ☐ No

C21. Have you ever sniffed glue, breathed the contents of aerosol spray cans, or inhaled any paints or sprays to get high?

- ☐ Yes
- ☐ No

C22. Have you ever taken **steroid pills or shots** without a doctor's prescription?

- ☐ Yes
- ☐ No

C. The next 4 questions ask about sexual intercourse

C23. Have you ever had sexual intercourse?

- ☐ Yes
- ☐ No

C24. How old were you when you had sexual intercourse for the first time?

- ☐ I have never had sexual intercourse
- ☐ 8 years old or younger
- ☐ 9 years old
- ☐ 10 years old
- ☐ 11 years old
- ☐ 12 years old
- ☐ 13 years old or older

C25. With how many people have you ever had sexual intercourse?

- ☐ I have never had sexual intercourse
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C26. The **last time** you had sexual intercourse, did you or your partner use a condom?

- ☐ I have never had sexual intercourse
- ☐ Yes
- ☐ No

This is the end of the survey. Thank you very much for your help.

Appendix 10

High School Risk Behavior Survey

You are currently enrolled in study of a recently approved quadrivalent HPV vaccine. The brand name of this vaccine is GARDASIL®. It is the first vaccine approved for use in pre-teens, teenagers and young adults to prevent human papillomavirs or HPV. HPV is a virus that is transmitted by sex and is associated with development of cancers of the anus and genital tract in both males and females. This survey is about health behaviors and your knowledge about HPV and human immunodeficiency virus (HIV- the virus that causes AIDS). It has been developed so you can tell us what you know about HPV and HIV and what you do that may affect your health. The information you give will be used to help us better understand how to provide better health education for young people like yourself.

The questions in the survey about health behaviors are identical to questions on the CDC's Youth Risk Behavior Survey that is administered nationally every 2 years to students across the United States attending public and private middle schools and high schools. The investigators conducting this quadrivalent HPV vaccine study have added additional specific questions about certain types of sexual behaviors, HPV and HIV because we are giving the vaccine to teens and young adults who are HIV positive as well as those who are HIV negative.

This is a web-based survey that will be administered via the internet. Similar to when you log on to "My Space" or other websites that have restricted access, you will need to decide on a unique USER ID (an alias). A password code will be provided to you to access and complete the survey over the internet.

You will NOT be asked to give your name. However you will be asked for background information (including your date of birth) and HIV status. It is important that you enter your date of birth accurately so the database can correctly calculate how old you are at the time you take this survey.

Important Things You Should Know:

- The answers you give will be kept private. No one will know what answer you select to any of the questions.
- Answer the questions based on what you really do.
- Completing the survey is entirely voluntary. Whether or not you answer the questions will not affect your participation in this vaccine study. *If you are not comfortable answering a question, just leave it blank.*
- The questions that ask about your background will be used only to describe the people who are completing this survey. The information will not be used to find out your name. *No names will ever be reported.*
- Make sure to read every question and then select the one answer that best describes what you think or what you do.
- When you are finished, follow the instructions of the study staff that helped you log on to the web site.

Thank you very much for your help.

A. Background Information

A1. Are you: ☐ Male ☐ Female

A2. What is your date of birth? ___ / ___ / _____
dd mm yyyy

A3. Are you Hispanic or Latino?
☐ Yes ☐ No

A4. What is your race? ***[Check <u>all</u> that apply]***
☐ Black or African American ☐ White
☐ American Indian or Alaskan Native ☐ Asian
☐ Native Hawaiian/other Pacific Islander ☐ Other _____

A5. Are you currently in school?
☐ Yes ☐ No

A5A. What grade are you in?
☐ Less than 6th grade
☐ 6th grade
☐ 7th grade
☐ 8th grade
☐ 9th grade
☐ 10th grade
☐ 11th grade
☐ 12th grade
☐ College or vocational school
☐ Graduate school

A5B. What was the highest level of education you completed?
☐ Middle school
☐ Some high school
☐ High school or equivalent (GED)
☐ Some college/A.A. degree/vocational training
☐ College degree/postgraduate

A6. Who do you live with?
☐ I live by myself
☐ I live with my biologic parents
☐ I live with my foster parents
☐ I live with my adoptive parents
☐ I live with other relatives
☐ I live with a roommate(s)
☐ I live with a boyfriend, girlfriend, or spouse
☐ Other (Specify: _____)

A7. Do you have HIV infection? ☐ Yes ☐ No

B. The next 11 questions are about human papillomavirus or HPV.

B1. Have you heard of human papillomavirus, or HPV?

- ☐ Yes
- ☐ No ⟶ ***Skip to question 12***

B2. How did you hear about it?

- ☐ A doctor, nurse or other health care provider
- ☐ Friend or family member
- ☐ Internet
- ☐ TV
- ☐ Magazine or newspaper
- ☐ Other (specify: _____)

B3. HPV is sexually transmitted.

- ☐ True
- ☐ False
- ☐ Don't know

B4. HPV is the main cause of cervical cancer.

- ☐ True
- ☐ False
- ☐ Don't know

B5. Who can carry HPV?

- ☐ Men
- ☐ Women
- ☐ Men and Women
- ☐ Neither men nor women
- ☐ Don't know

B6. Genital warts cause cervical cancer.

- ☐ True
- ☐ False
- ☐ Don't know

B7. The pill protects against HPV.

- ☐ True
- ☐ False
- ☐ Don't know
- ☐

B8. Condoms provide total protection against HPV.

- ☐ True
- ☐ False
- ☐ Don't know

B9. People can have HPV and not have any symptoms.

- ☐ True
- ☐ False
- ☐ Don't know

B10. A pap smear can tell you if you've been exposed to HPV.

- ☐ True
- ☐ False
- ☐ Don't know

B11. HPV can be transmitted by

- ☐ Oral sex
- ☐ Vaginal sex
- ☐ Anal sex
- ☐ Any genital contact (including fingers)
- ☐ All of the above
- ☐ None of the above
- ☐ Don't know

B. The next 26 questions are about human immunodeficiency virus or HIV.

B.	Can HIV can be spread through:	Yes	No	Don't know
B12.	Blood	☐	☐	☐
B13.	Vaginal fluids	☐	☐	☐
B14.	Saliva	☐	☐	☐
B15.	Semen	☐	☐	☐
B16.	Breast milk	☐	☐	☐
B17.	Skin contact	☐	☐	☐

	Can HIV be spread through any of the following behaviors?	Yes	No	Don't know
B18.	Kissing	☐	☐	☐
B19.	Oral sex	☐	☐	☐
B20.	Vaginal Sex	☐	☐	☐
B21.	Eating or drinking from common utensils	☐	☐	☐
B22.	Anal sex	☐	☐	☐
B23.	Living with an HIV-infected person	☐	☐	☐
B24.	Mosquitos	☐	☐	☐
B25.	Sharing needles with an HIV-infected person	☐	☐	☐
B26.	Needle stick injuries (a health care worker being stuck with an infected needle)	☐	☐	☐
B27.	Piercing or tattooing with non-sterile needles	☐	☐	☐
B28.	Sneezing or coughing	☐	☐	☐
B29.	Shaking hands	☐	☐	☐
B30.	Hugging	☐	☐	☐
B31.	Using the same restroom as an HIV-infected person	☐	☐	☐
B32.	Drinking from the same water fountain as an HIV-infected person	☐	☐	☐

	Can HIV can be transmitted by:	Yes	No	Don't know
B33.	An HIV-infected person with an undetectable level of virus	☐	☐	☐
B34.	An HIV-infected person with no symptoms	☐	☐	☐
B35.	An HIV-infected person who has not been diagnosed with AIDS	☐	☐	☐
B36.	An HIV-infected person who is taking medications	☐	☐	☐
B37.	An HIV-infected woman to her unborn child.	☐	☐	☐

C. The next 4 questions ask about safety.

C1. **When you rode a bicycle** during the past 12 months**,** how often did you wear a helmet?

- ☐ I did not ride a bicycle during the past 12 months
- ☐ Never wore a helmet
- ☐ Rarely wore a helmet
- ☐ Sometimes wore a helmet
- ☐ Most of the time wore a helmet
- ☐ Always wore a helmet

C2. How often do you wear a seat belt when **riding in** a car driven by someone else?

- ☐ I do not ride in a car driven by someone else
- ☐ Never
- ☐ Rarely
- ☐ Sometimes
- ☐ Most of the time
- ☐ Always

C3. During the past 30 days, how many times did you **ride** in a car or other vehicle **driven by someone who had been drinking alcohol?**

- ☐ I have never ridden in a car driven by someone else who had been drinking alcohol
- ☐ 0 times
- ☐ 1 time
- ☐ 2 or 3 times
- ☐ 4 or 5 times
- ☐ 6 or more times

C4. During the past 30 days, how many times did you **drive** a car or other vehicle **when you had been drinking alcohol?**

- ☐ I have never driven a car after drinking alcohol 0 times
- ☐ 0 times
- ☐ 1 time
- ☐ 2 or 3 times
- ☐ 4 or 5 times
- ☐ 6 or more times

C. The next 11 questions ask about violence-related behaviors.

C5. During the past 30 days, on how many days did you carry **a weapon** such as a gun, knife or club?

- ☐ 0 days
- ☐ 1 day
- ☐ 2 or 3 days
- ☐ 4 or 5 days
- ☐ 6 or more days

C6. During the past 30 days, on how many days did you carry **a gun?**

- ☐ 0 days
- ☐ 1 day
- ☐ 2 or 3 days
- ☐ 4 or 5 days
- ☐ 6 or more days

C7. During the past 30 days, on how many days did you carry a weapon such as a gun, knife or club **on school property?**

- ☐ 0 days
- ☐ 1 day
- ☐ 2 or 3 days
- ☐ 4 or 5 days
- ☐ 6 or more days

C8. During the past 30 days, on how many days did you **not** go to school because you felt you would be unsafe at school or on your way to or from school?

- ☐ 0 days
- ☐ 1 day
- ☐ 2 or 3 days
- ☐ 4 or 5 days
- ☐ 6 or more days

C9. During the past 12 months, how many times has someone threatened or injured you with a weapon such as a gun, knife or club **on school property?**

- ☐ 0 times
- ☐ 1 time
- ☐ 2 or 3 times
- ☐ 4 or 5 times
- ☐ 6 or 7 times
- ☐ 8 or 9 times
- ☐ 10 or 11 times
- ☐ 12 or more times

C10. During the past 12 months, how many times has someone stolen or deliberately damaged your property such as your car, clothing, or books **on school property?**

- ☐ 0 times
- ☐ 1 time
- ☐ 2 or 3 times

- ☐ 4 or 5 times
- ☐ 6 or 7 times
- ☐ 8 or 9 times
- ☐ 10 or 11 times
- ☐ 12 or more times

C11. During the past 12 months, how many times were you in a physical fight?

- ☐ 0 times
- ☐ 1 time
- ☐ 2 or 3 times
- ☐ 4 or 5 times
- ☐ 6 or 7 times
- ☐ 8 or 9 times
- ☐ 10 or 11 times
- ☐ 12 or more times

C12. During the past 12 months, how many times were you in a physical fight in which you were injured and had to be treated by a doctor or nurse?

- ☐ 0 times
- ☐ 1 time
- ☐ 2 or 3 times
- ☐ 4 or 5 times
- ☐ 6 or more times

C13. During the past 12 months, how many times were you in a physical fight **on school property?**

- ☐ 0 times
- ☐ 1 time
- ☐ 2 or 3 times
- ☐ 4 or 5 times
- ☐ 6 or 7 times
- ☐ 8 or 9 times
- ☐ 10 or 11 times
- ☐ 12 or more times

C14. During the past 12 months, did your boyfriend or girlfriend ever hit, slap, or physically hurt you on purpose?

- ☐ Yes
- ☐ No

C15. Have you ever been physically forced to have sexual intercourse when you did not want to?

- ☐ Yes
- ☐ No

C. The next 11 questions ask about tobacco use.

C16. Have you ever tried cigarette smoking, even one or two puffs?

- ☐ Yes
- ☐ No

C17. How old were you when you smoked a whole cigarette for the first time?

- ☐ I have never smoked a whole cigarette
- ☐ 8 years old or younger
- ☐ 9 or 10 years old
- ☐ 11 or 12 years old
- ☐ 13 or 14 years old
- ☐ 15 or 16 years old
- ☐ 17 years old or older

C18. During the past 30 days on how many days did you smoke cigarettes?

- ☐ 0 Days
- ☐ 1 or 2 days
- ☐ 3 to 5 days
- ☐ 6 to 9 days
- ☐ 10 to 19 days
- ☐ 20 to 29 days
- ☐ All 30 days

C19. During the past 30 days, on the days you smoked, how many cigarettes did you smoke **per day**?

- ☐ I did not smoke cigarettes during the past 30 days
- ☐ Less than 1 cigarette per day
- ☐ 1 cigarette per day
- ☐ 2 to 5 cigarettes per day
- ☐ 6 to 10 cigarettes per day
- ☐ 11 to 20 cigarettes per day
- ☐ More than 20 cigarettes per day

C20. During the past 30 days, how did you **usually** get your own cigarettes? Select only **one** response.

- ☐ I did not smoke cigarettes during the past 30 days
- ☐ I bought them in a store such as a convenience store, supermarket, discount store or gas station.
- ☐ 1 bought them from a vending machine.
- ☐ I gave someone else money to buy them for me.
- ☐ I borrowed (or bummed) them from someone else.
- ☐ A person 18 years old or older gave them to me.
- ☐ I took them from a store or family member.
- ☐ I got them some other way.

C21. During the past 30 days, on how many days did you smoke cigarettes **on school property**?

- ☐ 0 Days
- ☐ 1 or 2 days
- ☐ 3 to 5 days
- ☐ 6 to 9 days
- ☐ 10 to 19 days
- ☐ 20 to 29 days
- ☐ All 30 days

C22. Have you ever smoked cigarettes daily, that is, at least one cigarette every day for 30 days?

- ☐ Yes
- ☐ No

C23. During the past 12 months, did you ever try **to quit** smoking cigarettes?

- ☐ Yes
- ☐ No

C24. During the past 30 days, on how many days did you use **chewing tobacco, snuff, or dip,** such as Redman, Levi Garrett, Beechnut, Skoal, Skoal Bandits, or Copenhagen?

- ☐ 0 days
- ☐ 1 or 2 days
- ☐ 3 to 5 days
- ☐ 6 to 9 days
- ☐ 10 to 19 days
- ☐ 20 to 29 days
- ☐ All 30 days

C25. During the past 30 days, on how many days did you use **chewing tobacco, snuff, or dip on school property?**

- ☐ 0 days
- ☐ 1 or 2 days
- ☐ 3 to 5 days
- ☐ 6 to 9 days
- ☐ 10 to 19 days
- ☐ 20 to 29 days
- ☐ All 30 days

C26. During the past 30 days, on how many days did you smoke **cigars, cigarillos, or little cigars**?

- ☐ 0 days
- ☐ 1 or 2 days
- ☐ 3 to 5 days
- ☐ 6 to 9 days
- ☐ 10 to 19 days
- ☐ 20 to 29 days
- ☐ All 30 days

C. The next 6 questions ask about drinking alcohol. This includes drinking beer, wine, wine coolers, and liquor such as rum, gin, vodka, or whiskey. For these questions, drinking alcohol does not include drinking a few sips of wine for religious purposes.

C27. During your life, on how many days have you had at least one drink of alcohol?

- ☐ 0 days
- ☐ 1 or 2 days
- ☐ 3 to 9 days
- ☐ 10 to 19 days
- ☐ 20 to 39 days
- ☐ 40 to 99 days
- ☐ 100 or more days

C28. How old were you when you had your first drink of alcohol other than a few sips?

- ☐ I have never had a drink of alcohol other than a few sips
- ☐ 8 years old or younger
- ☐ 9 or 10 years old
- ☐ 11 or 12 years old

- ☐ 13 or 14 years old
- ☐ 15 or 16 years old
- ☐ 17 years old or older

C29. During the past 30 days, on how many days have you had at least one drink of alcohol?

- ☐ 0 days
- ☐ 1 or 2 days
- ☐ 3 to 5 days
- ☐ 6 to 9 days
- ☐ 10 to 19 days
- ☐ 20 to 29 days
- ☐ All 30 days

C30. During the past 30 days, on how many days did you have 5 or more drinks of alcohol in a row, that is, within a couple of hours?

- ☐ 0 days
- ☐ 1 day
- ☐ 2 days
- ☐ 3 to 5 days
- ☐ 6 to 9 days
- ☐ 10 to 19 days
- ☐ 20 or more days

C31. During the past 30 days, how did you **usually** get the alcohol you drank

- ☐ I did not drink alcohol during the past 30 days
- ☐ I bought it in a store such as a liquor store, convenience store, supermarket, discount store or gas station.
- ☐ 1 bought it at a restaurant, bar, or club.
- ☐ I bought it at a public event such as a concert or sporting event
- ☐ I gave someone else money to buy it for me
- ☐ Someone gave it to me
- ☐ I took it from a store or family member.
- ☐ I got it some other way.

C32. During the past 30 days, on how many days did you have at least one drink of alcohol **on school property?**

- ☐ 0 days
- ☐ 1 or 2 days
- ☐ 3 to 5 days

- ☐ 6 to 9 days
- ☐ 10 to 19 days
- ☐ 20 to 29 days
- ☐ All 30 days

C. The next 4 questions ask about marijuana use. Marijuana also is called grass or pot.

C33. During your life, on how many times have you used marijuana?

- ☐ 0 times
- ☐ 1 or 2 times
- ☐ 3 to 9 times
- ☐ 10 to 19 times
- ☐ 20 to 39 times
- ☐ 40 to 99 times
- ☐ 100 or more times

C34. How old were you when you tried marijuana for the first time?

- ☐ I have never tried marijuana
- ☐ 8 years old or younger
- ☐ 9 or 10 years old
- ☐ 11 or 12 years old
- ☐ 13 or 14 years old
- ☐ 15 or 16 years old
- ☐ 17 years old or older

C35. During the past 30 days, how many times did you use marijuana?

- ☐ 0 times
- ☐ 1 or 2 times
- ☐ 3 to 9 times
- ☐ 10 to 19 times
- ☐ 20 to 39 times
- ☐ 40 or more times

C36. During the past 30 days, how many times did you use marijuana **on school property**?

- ☐ 0 times
- ☐ 1 or 2 times
- ☐ 3 to 9 times

- ☐ 10 to 19 times
- ☐ 20 to 39 times
- ☐ 40 or more times

C. The next 9 questions ask about other drugs.

C37. During your life, how many times have you used **any** form of cocaine, including powder, crack, or freebase?

- ☐ 0 times
- ☐ 1 or 2 times
- ☐ 3 to 9 times
- ☐ 10 to 19 times
- ☐ 20 to 39 times
- ☐ 40 or more times

C38. During the past 30 days, how many times did you use **any** form of cocaine, including powder, crack, or freebase?

- ☐ 0 times
- ☐ 1 or 2 times
- ☐ 3 to 9 times
- ☐ 10 to 19 times
- ☐ 20 to 39 times
- ☐ 40 or more times

C39. During your life, how many times have you sniffed glue, breathed the contents of aerosol spray cans, or inhaled any paints or sprays to get high?

- ☐ 0 times
- ☐ 1 or 2 times
- ☐ 3 to 9 times
- ☐ 10 to 19 times
- ☐ 20 to 39 times
- ☐ 40 or more times

C40. During your life, how many times have you used **heroin** (also called smack, junk, or China White)?

- ☐ 0 times
- ☐ 1 or 2 times
- ☐ 3 to 9 times
- ☐ 10 to 19 times

- ☐ 20 to 39 times
- ☐ 40 or more times

C41. During your life, how many times have you used **methamphetamines** (also called speed, crystal, crank, or ice)?

- ☐ 0 times
- ☐ 1 or 2 times
- ☐ 3 to 9 times
- ☐ 10 to 19 times
- ☐ 20 to 39 times
- ☐ 40 or more times

C42. During your life, how many times have you used **ecstasy** (also called MDMA)?

- ☐ 0 times
- ☐ 1 or 2 times
- ☐ 3 to 9 times
- ☐ 10 to 19 times
- ☐ 20 to 39 times
- ☐ 40 or more times

C43. During your life, how many times have you taken **steroid pills or shots** without a doctor's prescription?

- ☐ 0 times
- ☐ 1 or 2 times
- ☐ 3 to 9 times
- ☐ 10 to 19 times
- ☐ 20 to 39 times
- ☐ 40 or more times

C44. During your life, how many times have you used a needle to inject any **illegal** drug into your body?

- ☐ 0 times
- ☐ 1 or 2 times
- ☐ 3 to 9 times
- ☐ 10 to 19 times
- ☐ 20 to 39 times
- ☐ 40 or more times

C45. During the past 12 months, has anyone offered, sold or given you an illegal drug **on school property?**

- ☐ Yes
- ☐ No

C. The next 26 questions ask about your sexual behavior. Remember that your answers are confidential. Everyone has different experiences. We are interested in what has happened in your life.

C46. Have you ever had sexual intercourse?

- ☐ Yes
- ☐ No

C47. How old were you when you had sexual intercourse for the first time?

- ☐ I have never had sexual intercourse
- ☐ 11years old or younger
- ☐ 12 years old
- ☐ 13 years old
- ☐ 14 years old
- ☐ 15 years old
- ☐ 16 years old or older
- ☐ 17 years old or older

C48. During your life, with how many people have you had sexual intercourse?

- ☐ I have never had sexual intercourse
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C49. During the past 3 months, with how many people have you had sexual intercourse?

- ☐ I have never had sexual intercourse
- ☐ 0 persons
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people

- ☐ 5 people
- ☐ 6 or more people

C50. Did you drink alcohol or use drugs before you had sexual intercourse the **last time?**

- ☐ I have never had sexual intercourse
- ☐ Yes
- ☐ No

C51. The **last time** you had sexual intercourse, did you or your partner use a condom?

- ☐ I have never had sexual intercourse
- ☐ Yes
- ☐ No

C52. The **last time** you had sexual intercourse, what one method did you or your partner use to **prevent pregnancy**? (Select only **one** response.)

- ☐ I have never had sexual intercourse
- ☐ No method was used to prevent pregnancy
- ☐ Birth control pills
- ☐ Condoms
- ☐ Depo-Provera (injectable birth control)
- ☐ Withdrawal
- ☐ Some other method
- ☐ Not sure

C53. During your life, have you ever had digital-genital contact with anyone (where your or your partner's hands touched the other person's genitals)?

- ☐ Yes
- ☐ No

C54. How many people have you had digital-genital contact with in the past **12 months**?

- ☐ I have never had digital-genital contact
- ☐ 0 persons
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C55. Of the people that you have had digital-genital contact with in the past 12 months, **how many were men**?

- ☐ I have never had digital-genital contact
- ☐ I have never had digital-genital contact **with men**
- ☐ 0 persons
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C56. Of the people that you have had digital-genital contact with in the past 12 months, **how many were women**?

- ☐ I have never had digital-genital contact
- ☐ I have never had digital-genital contact **with women**
- ☐ 0 persons
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C57. During your life, have you ever had oral sex?

- ☐ Yes
- ☐ No

C58. How many people have you oral sex with in the past **12 months**?

- ☐ I have never had oral sex
- ☐ 0 persons
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C59. Of the people that you had oral sex with in the past 12 months, **how many were men**?

- ☐ I have never had oral sex
- ☐ I have never had oral sex **with men**
- ☐ 0 persons
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C60. Of the people that you oral sex with in the past 12 months, **how many were women**?

- ☐ I have never had oral sex
- ☐ I have never had oral sex **with women**
- ☐ 0 persons
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C61. How often did you **use a condom** when you had oral sex in the past 12 months?

- ☐ I have never had oral sex
- ☐ I have not had oral sex in the past 12 months
- ☐ Never used a condom
- ☐ Used a condom less than half the time
- ☐ Used a condom about half the time
- ☐ Used a condom more than half the time
- ☐ Always used a condom

C62. During your life, have you ever had vaginal sex (sexual intercourse)?

- ☐ Yes
- ☐ No

C63. How many people have you vaginal sex with in the past **12 months**?

- ☐ I have never had vaginal sex
- ☐ 0 persons

- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C64. Of the people that you had vaginal sex with in the past 12 months, **how many were men**?

- ☐ I have never had vaginal sex
- ☐ I have never had vaginal sex **with men**
- ☐ 0 persons
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C65. Of the people that you vaginal sex with in the past 12 months, **how many were women**?

- ☐ I have never had vaginal sex
- ☐ I have never had vaginal sex **with women**
- ☐ 0 persons
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C66. How often did you **use a condom** when you had vaginal sex in the past 12 months?

- ☐ I have never had vaginal sex
- ☐ I have not had vaginal sex in the past 12 months
- ☐ Never used a condom
- ☐ Used a condom less than half the time
- ☐ Used a condom about half the time
- ☐ Used a condom more than half the time
- ☐ Always used a condom

C67. During your life, have you ever had anal sex?

- ☐ Yes
- ☐ No

C68. How many people have you anal sex with in the past **12 months**?

- ☐ I have never had anal sex
- ☐ 0 persons
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C69. Of the people that you had anal sex with in the past 12 months, **how many were men**?

- ☐ I have never had anal sex
- ☐ I have never had anal sex **with men**
- ☐ 0 persons
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C70. Of the people that you anal sex with in the past 12 months, **how many were women**?

- ☐ I have never had anal sex
- ☐ I have never had anal sex **with women**
- ☐ 0 **persons**
- ☐ 1 person
- ☐ 2 people
- ☐ 3 people
- ☐ 4 people
- ☐ 5 people
- ☐ 6 or more people

C71. How often did you **use a condom** when you had anal sex in the past 12 months?

- ☐ I have never had anal sex
- ☐ I have not had anal sex in the past 12 months

- ☐ Never used a condom
- ☐ Used a condom less than half the time
- ☐ Used a condom about half the time
- ☐ Used a condom more than half the time
- ☐ Always used a condom

This is the end of the survey. Thank you very much for your help.

Appendix 11

Protocol Summary and Overview for Informational and Recruitment Purposes

NIH Clinical Trial- Bethesda, Maryland

A Study of Quadrivalent Human Papilloma Virus (HPV) Vaccine in HIV-Infected and HIV-Negative Pre-Adolescents, Adolescents and Young Adults

HIV positive and healthy, HIV negative subjects 12 to 26 years of age are needed as volunteer human research subjects for a study to evaluate the immunogenicity of the quadrivalent human papilloma virus (types 6, 11, 16, 18) recombinant vaccine. This vaccine was approved and licensed by the FDA for clinical use on June 8, 2006 and is marketed under the name GARDASIL®. In healthy, HIV-negative, women, vaccination with GARDASIL® induces immune responses i.e. neutralizing antibody titers to the four HPV virus types that are in the vaccine: types 6, 11, 16, and 18. These HPV types are associated with the development of genital warts and precancerous lesions of the cervix and cervical cancer. The vaccine was demonstrated to be highly (100%) effective in preventing the conditions listed above in individuals with normal immune systems. However, it is not known whether the vaccine will be as effective in generating antibody responses or if the responses will last as long, in individuals whose immune systems are compromised, such as individuals with HIV-infection. Since individuals with HIV-infection are at greater risk of becoming infected with HPV and developing pre-cancerous complications from it, both females and males are being included in this study. HIV negative subjects are being studied at the same time as HIV positive subjects in order to directly compare how well the vaccine is able to induce antibodies compared to a healthy age-matched population. The study is four years long and involves 7 study visits the first year and then 1 study visit every six months (2 visits per year) during years 2 through 4.

Persons with HIV-infection must have greater than 350 CD4 cells/mm^3 and a viral load $\leq$ 20,000 copies/ml. They may be on HAART therapy or no antiretroviral treatment. Healthy, normal volunteers will have to agree to undergo HIV testing to participate in the study. Study-related screening and diagnostic tests will be performed at the NIH Clinical Research Center in Bethesda, MD without charge. Quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine will be provided free to all study subjects. For volunteers who live > 50 miles from the NIH, transportation costs will be provided after the first visit if the individual is found to be eligible and enrolls in the study.

For more information, volunteers and/or physicians should contact the National Cancer Institute Clinical Studies Support Center toll free at 1-888-NCI-1937 or at http://bethesdatrials.cancer.gov.

Additional Informational Materials for Study Recruitment Purposes

Double-Sided Tri-Folder Flyer: **ARE YOU PROTECTED…?**

Front of Flyer

ARE YOU PROTECTED

...from the sexually transmitted infection HPV (Human Papillomavirus)

?

If you are an **HIV positive** female OR male, **between 12 and 26 years of age, and HAVE NOT YET RECEIVED an HPV Vaccine,** call us about participating in a Clinical Research Trial.

Someone who is already infected with HIV is at greater risk for infection with HPV, and may develop complications from this virus.

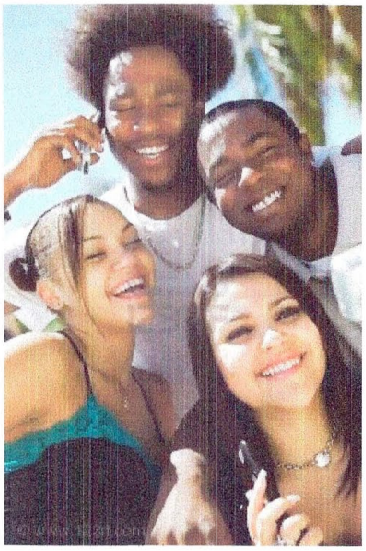
If you are a **healthy** male OR female, **between 12 and 26 years of age, and HAVE NOT YET RECEIVED an HPV Vaccine,** call us about participating in a Clinical Research Trial.

Back of Flyer

Enrolled volunteers must be **between 12 and 26 years of age**, and will come to the NIH for **FREE** study visits approximately once a month for 7 months, with follow-up visits twice a year for up to 4 years.

All enrolled volunteers will receive the Gardasil® HPV Vaccine and study-related medical care and diagnostic testing as part of participation in the study.

NATIONAL CANCER INSTITUTE



Vaccination with Quadrivalent GARDASIL® was demonstrated to be highly effective in preventing the development of:

Genital Warts and Anal Cancer

- in men and women

Precancerous Lesions of the cervix **and Cervical Cancer** - in women

You are invited to take part in a research study at the National Cancer Institute / National Institutes of Health. Taking part in NIH research is completely voluntary. If you are under the age of 18, a parent or guardian must also consent to your participation in this research study.

WE NEED YOUR HELP – E-mail or call the Vaccine Branch of NCI for details.

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301-435-4733