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Incidence, Persistence, and Recurrence of Anogenital α- Mucosal HPV Infections (HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58)

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by

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DEDICATION

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ABSTRACT

Objectives: The aims of this study were to: 1) Assess whether naturally induced anti-HPV antibodies are associated with subsequent acquisition of genital HPV 6, 11, 16, and 18 infections in men, 2) assess the recurrence (redetection) of genital HPV infections of the 9-valent vaccine HPV types and investigate factors associated with recurrent infections among men, and 3) assess the risk of type-specific sequential acquisition of anal HPV infection following a genital HPV infection of the 9-valent vaccine HPV types among men who have sex with women (MSW).

Methods: 4,123 healthy men were followed every six months (median follow-up time 4.1 years). HPV antibodies were measured at baseline using a virus-like particle-based ELISA assay. Genital and anal HPV genotypes were detected using the Roche Linear Array assays. Kaplan-Meier curves and Cox models were developed to assess associations between serum anti-HPV antibody and subsequent incident HPV infections. Individual type analyses and grouped analyses were carried out to assess type-specific recurrence of the 9-valent vaccine HPV types. Risk of sequential anal HPV infection was assessed by examining incident rate ratios (IRR) and adjusted hazard ratios (aHR) among men with a prior genital HPV infection compared to men without a prior genital HPV infection.

Results: 1) Significantly higher rates of incident infections were observed for HPV 16 among baseline HPV 16 seropositive men (aHR 1.37, 95% CI 1.01-1.86). Risk of persistent HPV 18

infection was significantly lower among HPV 18 seropositive men in unadjusted models, but not in the adjusted model, while incident and six-month persistent HPV 6 and 11 infections did not differ by baseline serostatus. 2) Up to 31% of prior prevalent and 20% of prior incident HPV infections recurred over time in individual type analyses. New female sexual partners, frequency of sexual intercourse with female partners, and new male sexual partners were associated with type-specific recurrence of HPV infections (HPV 6, 16, 31 and 58). In grouped analyses, lifetime number of male sexual partners (aOR = 2.40, 95% CI 1.19-4.84) and number of new male sexual partners (aOR 2.35, 95% CI 1.16-4.74) were associated with recurrence of HPV infections. 3) In individual type analyses, men with a prior HPV 16 genital infection had a significantly higher risk of subsequent anal HPV 16 infection (aHR=4.63, 95% CI 1.41-15.23). Significantly higher HRs were observed for any of the nine HPV types (aHR= 2.8, 95% CI1.32-5.99), high risk HPV types (aHR=2.65, 95% CI 1.26, 5.55) and low risk HPV types (aHR=5.89, 95% CI1.29, 27.01) in grouped analyses.

Conclusion: Baseline seropositive status among men was not associated with a reduction in subsequent incident genital HPV 6, 11, and 16 infections, but with a possible protective effect for persistent HPV 18 infections. Men are also susceptible to recurrence of type-specific genital HPV infections, and recurrence of HPV infection was associated with high-risk sexual behaviors. MSW men with prior genital HPV infections are more likely to have a subsequent type-specific anal HPV infection than men who did not have prior genital HPV infections. Understanding the natural history of HPV infections among men is essential to control HPV associated diseases in both men and women.

INTRODUCTION

Human papillomavirus (HPV) is one of the most common sexually transmitted infections in men and women in the U.S. (1). HPV infection causes multiple cancers among men and women, including cancers of the cervix, vagina, vulva, penis, oral cavity, head and neck, and anal canal (2). The role of HPV in the etiology of cervical cancer has been well documented, and vaccines targeting the most common HPV types (HPV 6, 11, 16 and 18) have been in use since 2006.[3] Despite low uptake in the U.S., use of HPV vaccination has led to reduced prevalence of HPV infections and premalignant conditions (3,4). Until recently, vaccination efforts have been largely targeted toward girls and young women. The natural history of HPV infection among men was not studied in detail until recently. It is now known that HPV infection in men plays an important role in the etiology of penile, anal and oropharyngeal cancers. Furthermore, HPV has been shown to be readily transmitted between sexual partners (5). Thus, an understanding of HPV infection in men is critical for preventing HPV-related cancers among both men and women.

In the proposed study, we plan to examine the association between serum anti-HPV antibodies and subsequent incident infections for specific HPV types. The association between anti-HPV antibodies and incident HPV infection has been studied among women, but similar associations are not clearly understood among men. We will also assess recurrent HPV infections, factors affecting recurrence for specific HPV types, and genital-anal genotype-

concordant HPV infections. For the proposed study, we will utilize data from the HPV Infection in Men (HIM) Study. The HIM study is a natural history study of HPV in more than 4,000 healthy men recruited between 2005-2009 from the U.S., Brazil, and Mexico. Findings from the proposed study will provide valuable information about the natural immune response to HPV, recurrent HPV infections and genital-anal genotype-concordant HPV infections will be useful in developing prevention strategies, such as HPV vaccination targeting men.

Background and significance

HPV is the most common sexually transmitted infection in the world and was responsible for about 527,624 new cervical cancers that occurred worldwide in 2012 (6, 7). In the U.S., 32,000 cancer cases in men and women were attributed to HPV in 2009, with the cost for prevention and treatment of HPV associated diseases estimated to be $8 billion per year (7, 8). An estimated 79 million people are currently infected with HPV, and 14 million individuals acquire new HPV infections each year in the U.S. (9). More than 50% of sexually active individuals are infected with one or more types of HPV at some point in their lives (10).

HPVs are a group of more than 150 related viruses, with about 40 distinct types that infect the genital area (11). HPV infections can be broadly classified as mucosal (α genus) and cutaneous, (including α, β, γ, μ (Mupa), and Nv (Nupa) genera) (12). Alpha (α) HPV genus (including HPV 6, 11, 16, 18, 31, 33, 45, 52, 58 etc) infections can lead to the development of various cancers (cervix, vulva, vagina, penis, and oropharynx), as well as benign lesions such as genital warts and respiratory papillomatosis (13, 14). HPV 16 and 18 are the most common oncogenic (high-risk) types and account for about 70% of cervical cancers, 85% of anal cancers, and 47% of penile cancers (15, 16). HPV 6 and 11 are the most common non-oncogenic (low-risk) types and account for about 90% of genital warts (15). HPV DNA is detected in

approximately 88- 94% of anal cancers (24, 25), 29-82% of penile carcinoma cases (19, 20), 70-100% of penile intraepithelial neoplasia (PeIN) (18), and 80-100% of genital warts (22, 23). Among men, genital HPV prevalence has been reported to be as high as 73% (17). In 2007, WHO (IARC) concluded that HPV 16 infection is a class 1A carcinogen for the development of penile, anal and oropharyngeal cancers (14). According to 2007-2011 SEER data, incidence rates for oropharyngeal, anal and penile cancers were 11.0 per 100,000, 1.8 per 100,000 and 1.0 per 100,000, respectively; with an increasing number of cases occurring due to HPV infection in the U.S. (18).

Immunization with one of the approved HPV vaccines provides an important opportunity for prevention of HPV infection and associated diseases in men and women. The three currently available options include the bivalent (protecting against HPV 16 and 18), quadrivalent (HPV 6, 11, 16, and 18), and recently approved 9-valent (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58) vaccines (26, 27). Although only one-third of girls aged 13-17 years received all three recommended doses of an HPV vaccine since the first HPV vaccine approval in 2006, the prevalence of vaccine type HPVs has decreased by 56% among females in this age group, highlighting the potential to eliminate the HPV types responsible for most HPV-related disease should higher rates of vaccine dissemination be achieved (3). Furthermore, a recent study observed a reduced prevalence of HPV 16/18 type in high grade cervical intraepithelial neoplasia (CIN2/3) among vaccinated women (4).

Vaccination leads to a robust serum antibody response that is associated with protection against future anogenital HPV infections and HPV-associated precancerous and cancerous lesions (28). Virus-like particle (VLP)-based ELISA assays have been used to measure serum HPV antibodies, which correlate well with protection against incident viral infection (29). HPV

antibodies are also produced following natural infection but at lower levels, particularly in men (29-32). While these antibodies from natural infection are likely markers of moderate immunity against subsequent infections and precancerous lesions among women (33- 35), the few studies conducted to date do not indicate a protective effect among men (30-32). While studies have examined recurrence (redetection) of HPV infection among women (36-39), to our knowledge no previous study has examined the recurrence of HPV infections among men. Similarly only few studies have examined the cervical and anal genotype-concordant HPV infections among women (44-46). To the best of our knowledge, studies examining sequential acquisition of genital and anal genotype-concordant infections, among men who have sex with women (MSW), have not been conducted.

In the current study, we plan to evaluate the baseline serum antibody status for α-mucosal HPV types (6, 11, 16, and 18) and their role in subsequent incidence and persistence of HPV infection among men. We will also assess the concordance between genital and anal HPV infections for 9- vaccine type HPVs (HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58) among MSWs. Findings from this study will help shed light on natural immunity against HPV in men and factors associated with persistence and recurrence of HPV infection. These results will be helpful to promote the HPV vaccination and increase utilization among men as well as women.

Specific aims and objectives

The purpose of this study is to assess the incidence, persistence, and recurrence of genital α-mucosal HPV infections (HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58) and evaluate whether HPV antibody status is associated with the natural history of these infections. We plan to accomplish these goals by pursuing the following specific aims:

Aim 1: Examine the association between baseline serum α-mucosal anti-HPV antibodies (HPV 6, 11, 16 and 18) and subsequent incidence and persistence of incidentally acquired type-specific genital HPV infection among men aged 18-70 years in the HIM study cohort.

Objective 1.1 - Determine the incidence rates of genital HPV 6, 11, 16 and 18 infections among baseline seronegative and seropositive subjects.

Hypothesis 1.1 - Our hypothesis is that incidence rates do not differ by baseline HPV serostatus.

Objective 1.2 - Determine the rate of persistence of incidentally acquired infections, with duration of 6 months or more, for genital HPV 6, 11, 16 and 18 infections among baseline seronegative and seropositive subjects.

Hypothesis 1.2 - Our hypothesis is that rates of persistent infections do not differ by baseline HPV serostatus.

Aim 2: Estimate the rate of recurrent genital α-mucosal HPV infections (HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58) among men aged 18-70 years, and the factors associated with recurrent infections.

Objective 2.1 - Estimate the rate of recurrent genital HPV infections for α-mucosal HPV vaccine types (HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58) among 4,123 men in the HIM study participants.

Hypothesis 2.1 - Our hypothesis is that the recurrence rates are lower than 10% for mucosal α-HPVs.

Objective 2.2 – Investigate factors associated with recurrent infections among 4,123 men in the HIM study for genital HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58.

Hypothesis 2.2 - Our hypothesis is that risk of recurrent infections will be higher among men aged 18-30 years, those from Brazil, current smokers, men who consume alcohol more than two drinks per day and those with riskier sexual behaviors (≥18 lifetime sexual partners, and ≥2 recent sexual partners).

Aim 3: Examine sequential genital and anal genotype-concordant HPV infections (HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58) and identify factors associated with anal HPV infections

following a prior genotype-concordant genital HPV infection among men who have sex with women (MSW).

Objective 3.1 – Identify sequential genital and anal genotype-concordant HPV infections for HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58.

Hypothesis 3.1 - Our hypothesis is that anal HPV infections are more likely to occur among MSW men who had a prior genotype-concordant (same genotype) genital HPV infection compared to men with no-prior genital infection.

Objective 3.2 - Investigate factors associated with an incident anal HPV infection following a prior genotype-concordant genital HPV infection, for HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58.

Hypothesis 3.2 - Our hypothesis is that anal HPV infection following genital HPV infection occurs due to auto-inoculation and therefore is not associated with sex with female partners among MSW.

REVIEW OF LITERATURE

Human papillomavirus (HPV)

World statistics:

Worldwide prevalence of HPV among women without cervical abnormalities is 11.7%, with the most prevalent genotypes being HPV 16 (3.2%) and HPV 18 (1.4%) (5). Higher prevalence has been observed in sub-Sahara Africa (24%), Eastern Europe (21%) and Latin America (16%). The prevalence increases sharply among women with cervical pathology, reaching approximately 90% in grade 3 intraepithelial neoplasia (5). Among men, HPV prevalence ranging from 1.3%-72.9% has been documented in various studies (14).

Cervical cancer is the second most common cancer among women aged 15-44 years, with approximately 528,000 new cervical cancers cases and 266,000 deaths occurring annually in the world (47). Almost all cervical cancers are caused by HPV infection, with HPV 16 and 18 responsible for 70% of these cancers (11). Apart from cervical cancers, HPV also causes anal, vulvar, vaginal, penile, and oropharyngeal cancers. According to a WHO 2013 publication, worldwide numbers of new cases for HPV-related cancers were as follows: 27,000 anal cancers, 27,000 vulvar cancers 13,000 vaginal cancers, and 26,000 penile cancers (21, 47). Penile cancer is more common in some parts of Asia, Africa and South America, and accounts for up to 10% of cancer cases among men (47). Worldwide annual age-adjusted incidence and mortality rates

for oropharyngeal cancer (OPC) are 5.9 and 3.3 per 100,000 persons, respectively (47). In developed countries, HPV associated OPC incidence rates are increasing among men and HPV 16 type has been implicated in most cases (48, 49).

U.S. statistics:

Each year 26,000 new cancer cases (17,000 in women and 9,000 in men) are attributable to HPV in the U.S. (50). Cervical cancer is the fourth most common cancer among women aged 15-44 years in the U.S., and about 3.9% women in the general population have HPV 16/18 infection at any given time (51). Annual incidence and mortality rates for cervical cancer are 7.8 and 2.3 per 100,000 women based on SEER 2007-2011 data (15), resulting in approximately 13,000 new cervical cancer cases and 6,600 cervical cancer deaths each year (51). For vulvar cancers, incidence and mortality rates are 2.4 and 0.5 per 100,000 women, respectively (15). Anal cancers are also associated with HPV infection with annual incidence and mortality rates of 1.8 and 0.2 per 100,000 in men and women (15). Penile cancers are rare in the U.S. and they account for less than 1% of cancers diagnosed among men (15). Oropharyngeal cancer (OPC) annual incidence in the U.S. is 10.6 cases per 100,000 persons, with an annual mortality rate of 2.5 per 100,000 persons (15). OPC incidence rates are about 4 times higher in men compared to women (52). Approximately 45,780 new cases and 8,650 deaths were attributed to OPC in the US in 2014 (53).

HPV classification

HPV is a non-enveloped double-stranded DNA virus that primarily infects stratified epithelia (54). HPV infections can be classified as mucosal (genus) and cutaneous, which include α, β, γ, μ (Mupa), and Nv (Nupa) genera (55). These genera differ according to the

primary site of infection (Table 1) (56-58). The α-HPV genus includes the most important HPV types associated with cervical, vulvar, vaginal, penile, anal, and oropharyngeal cancers and genital warts; including HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 (56). Alpha HPV genotypes can be further classified as high-risk (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68, 69, and 82) or low-risk (HPV 6, 11, 40, 42, 43, 44, 54, 61, 72, and 81), based on their oncogenicity (57). Most HPV infections (about 90%) are asymptomatic and resolve within two years, but persistent HPV infections can cause a variety of cancers and benign diseases (59).

Table 1: Different genera of papillomavirus family with HPV types and type of tissues affected (58)

Genus	**HPV type**	**Tissue tropism**
Alpha papillomavirus	6, 11, 16, 18, 26, 30, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 74, 81, 83, 84, 85, 86, 87, 89, and 90 7, 40, 43 and c91 2, 3, 10, 27, 38, 29, 57, 77, 78 and 94	Mucosal Mucosal and cutaneous Cutaneous
Beta papillomavirus	5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 75, 76, 80, 92, 93 and 96	Cutaneous
Gamma papillomavirus	4, 48, 50, 60, 65, 88 and 95	Cutaneous
Muppa papillomavirus	1 and 63	Cutaneous
Nupa papillomavirus	41	Cutaneous

Source: Reproduced from the publication by Koning et al (2008)[58].

Mechanism of infection

HPVs infect keratinocytes in the basal cell layer of the stratified epithelium, which is the only tissue in which they can replicate (60). HPVs replicate in the nucleus of keratinocytes in a differentiation-dependent manner, meaning that viral gene replication and transcription are tightly controlled by keratinocyte differentiation (60). Expression of HPV viral genes leads to expression of six nonstructural viral regulatory proteins (E1, E2, E4, E5, E6 and E7) and two structural viral capsid proteins (L1 and L2) (61). The E6 and E7 viral proteins are oncogenes, leading to deactivation of p53 and pRb tumor suppressor proteins (61). Various HPV protein encoding genes and organization of the HPV genome are shown in Figure 1 (62).

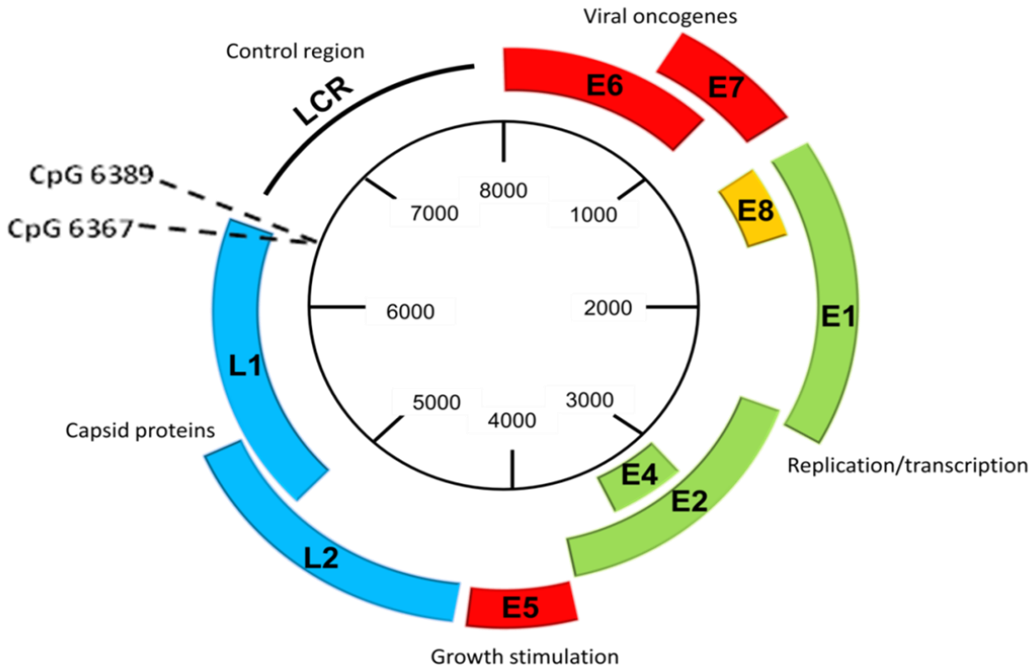

Figure 1: Genomic organization of high-risk mucosal HPV virus (62)

Source: Used with permission of Dr. Anna Giuliano, from the HIM Study presentation materials.

As shown in the figure, the HPV viral genome contains early (E) regions and late (L) regions, which indicate the position of the gene and their timing of expression in the viral life cycle (61, 62). E1 and E2 proteins help with viral replication and transcription, while E4 helps with viral release. E6 and E7 are major transforming proteins of the oncogenic HPVs and also have roles in the normal viral cell cycle (62). E5 protein has a role in growth stimulation and immune evasion (61, 62). Among the late region proteins, L1 is a major capsid protein and L2 is a minor capsid protein. The L1 major capsid protein binds with the basement membrane heparan sulfate proteoglycans (HSPGs), which are exposed due to any previous trauma to the skin (60). Gene replication and transcription are controlled by the upstream regulatory region (URR), which also contains promoter and enhancer regions (60-62).

The cell-mediated immune (CMI) response is responsible for clearing HPV-associated infection and benign lesions and is accompanied by a CD4+ T cell-dominated response (62). Failure of an effective CMI response, followed by high-risk HPV infection, can lead to persistent infection and increased probability of high grade intraepithelial neoplasia and invasive carcinoma (62). HPV infection effectively evades innate immune recognition, due to the fact that HPV is exclusively an intra-cellular infection; there is an absence of viremia, virus-induced cell death, and replication-associated inflammation (62). HPV also downregulates innate immune signals; which leads to inhibition of type I interferons (IFN-α and IFN-β), lack of signals for Langerhans cell activation/migration, and lack of recruitment of stromal dendritic and macrophage cells (62). Evasion of these immune responses allows infections to persist for months to several years and it may lead to high-grade intraepithelial neoplasia and upregulation of E6 and E7 proteins, which further suppresses the immune response (62). After HPV infection, non-dividing epithelial cells retain a normal active cell cycle, which may result in thickened,

exophytic lesions (60). Virus is released as the epithelial cells exfoliate. In cells that do not senesce, the viral genome integrates into the host chromosome which is associated with neoplastic progression (60).

As indicated previously, HPV infection and lesions are primarily targeted by CMI (60-62), with antibody response (humoral response) shown to follow the CMI response (62). Unlike the antibody response following infection with other viruses, lower antibody levels are detected in both animals and humans, which may be due to HPV's ability to evade the innate immune recognition (61, 62). As viral particles are released at the surface of the epithelium (rather than in blood or lymph nodes) as cell exfoliates and there is no viremia, circulating immune cells are not exposed to HPV. The humoral response is initiated in lymph nodes and this may be one of the explanations for lower antibody levels (62). Furthermore, current methods for serum antibody measurement may not be sensitive enough to detect low antibody levels and this may explain different findings observed in previous studies (31, 62).

HPV vaccines

Three HPV vaccines are currently approved for use in the U.S. A quadrivalent vaccine (Gardasil, HPV 6, 11, 16, 18) approved in 2006 (63,64), a bivalent vaccine (Cervarix; HPV 6 and 11) approved in 2009 (65), and a nonavalent vaccine (Gardasil 9, HPV 6, 11, 16, 18, 31, 33, 45, 52, 58) approved in 2014 (66). Among the types covered by these vaccines, HPV 16 and 18 are responsible for about 64% of invasive cancers, 70% of cervical cancers; while HPV 6 and 11 cause 90% of genital warts and most cases of recurrent respiratory papillomatosis (63, 66). These vaccines include virus-like particles (VLPs), which are prepared from the recombinant L1 capsid protein of HPV, and are therefore not live vaccines (63). HPV vaccine is recommended

starting at age 11-12 years for routine vaccination (67). The Advisory Committee on Immunization Practices (ACIP) of the CDC also recommends vaccination for 13-26 year old females and 13-21 year old males not vaccinated previously (66). These recommendations are extended to include men up to 26 years of age who are considered to be at higher risk of HPV acquisition (men who have sex with men or who are immune-compromised) (66).

In December 2014 a 9-valent vaccine (Gardasil-9) covering 5 additional HPV types (31, 33, 45, 52 and 58), along with quadrivalent HPV types (HPV 6, 11, 16 and 18) was approved in US (66). The 5 additional HPV strains are responsible for about 10% of HPV associated cancers and about 15% of cervical cancers (66). In a phase III clinical trial, the 9-valent vaccine demonstrated 96.7% efficacy in the prevention of cervical intraepithelial neoplasia grade 2, vulvar intraepithelial neoplasia grade 2 or 3, and vaginal intraepithelial neoplasia grade 2 or 3 caused by HPV 31, 33, 45, 52, or 58 (66).

According to a 2014 report, vaccine coverage for HPV among adolescent girls in the U.S. was 57.3% for one or more doses and 37.6% for all three doses (68). Coverage among adolescent boys was much lower, with 34.6% coverage for one or more doses and 13.9% for all three doses (68). HPV vaccination coverage among adolescents has not increased in recent years, and the coverage rates are far below the Healthy People 2020 target of 80% coverage for both adolescent girls and boys (68). Australia was the first country to launch a government-sponsored HPV vaccination program, and between 2007-2009, approximately 83% girls aged 12-17 years received at least one dose of the quadrivalent HPV vaccine, and 70% completed all three-dose series (69). Four years after initiation of this vaccination program, there was a significant reduction in the prevalence of vaccine-type HPVs (6, 11, 16, and 18) (69). Reductions in HPV-associated diseases and pre-malignant lesions in vaccinated populations have

also been documented in Australia, Denmark, and the U.K. (70-72). Thus, increased efforts to expand HPV vaccination coverage are required to prevent HPV- associated diseases in the U.S.

HPV infection among men

Although HPV natural history studies originally focused exclusively on women, the importance of HPV etiology and diseases among men has increasingly been recognized and studied. One such study, the HPV Infection in Men (HIM) Study is a prospective natural history study conducted among healthy men from three different countries (U.S., Brazil, and Mexico) (2). The HIM Study was the first large study to report the prevalence and type distribution of HPV among men residing in the USA, Brazil and Mexico (2). The initial findings of the HIM Study reported an overall HPV prevalence of 65.2%, with the highest prevalence in Brazil (72.3%), followed by Mexico (61.9%) and the U.S. (61.3%) (2). A systematic review in 2006 detected HPV prevalence ranging from 1.3%-72.9% among men, with more than 56% studies reporting prevalence over 20 % (14) with HPV 16 being the most commonly detected type. Five cross-sectional studies described young age at first sexual intercourse, greater number of lifetime and recent sexual partners, and high frequency of intercourse as risk factors for HPV infection in men (73-76). The HIM study reported an incidence rate of 38.4 per 1,000 person month (95% CI 34.3-43.0) for new genital HPV infections (8). For oncogenic HPV infections, this study reported hazards ratio (HR) of 2.40 (95% CI 1.38-4.18) for at least 50 partners vs. not more than one partner and HR of 2.57 (95% CI 1.46-4.49) for at least three male partners vs. no recent partners (8). Average duration of an HPV infection was 7.5 months and for HPV 16 was 12.2 months (8). Earlier cross-sectional and longitudinal studies also indicated that circumcision and condom use were associated with reduced risk of HPV infection among men (14, 73-78). The HIM study also showed that men have a consistent risk of HPV across the lifespan (ages 18-70

years) (13, 81). Oncogenic HPV detection was independently associated with lifetime and recent number of sexual partners, while non-oncogenic HPV infection was associated with only lifetime number of sexual partners (82). A few studies have found that smoking is a possible risk factor, while others found no association (83-85).

Previous literature

HPV antibodies and incident infection

Among women

Various studies among women have assessed the effect of anti-HPV antibodies on subsequent risk of incident infection acquisition. High levels of HPV 16 IgG antibodies were shown to be associated with reduced incidence of subsequent infection with HPV16 and related types (31, 33, 35, 52, and 58) (86). Persistent antibodies to HPV 16 ($p = 0.02$), HPV31 ($p<0.001$), HPV33 ($p=0.03$), HPV35 ($p=0.002$), HPV52 ($p=0.007$), HPV45 ($p=0.003$), and HPV53 ($p=0.01$) were also found to be associated with reduced risk of HPV incidence (87). Further studies also have shown protection against HPV infection among seropositive individuals (33-35, 88, 89). Although three studies did not find any association between anti-HPV antibodies and subsequent protection against incident type-specific HPV infection (90-92), the majority of studies indicated a moderate protective effect of anti-HPV antibodies against subsequent infections among women. Various studies have indicated a higher seroprevalence of anti-HPV antibodies among women compared with men for both oncogenic and non-oncogenic HPV types (93-97). Factors including differential immune response, serum antibody cut-off levels, differences in assay techniques, and different durations of antibody levels have been suggested for the difference in antibody response observed among women in these studies.

Among men

Only three studies have previously assessed the association between HPV antibodies and subsequent incident infection in men (29-31). A study conducted in HPV Infection in Men study cohort Tucson-Arizona, did not show a protective effect for HPV 16 and 18 antibodies (29), but this study had limited follow-up time, sample size, and availability of quantitative assessments for serum antibody levels. A study based on 2,187 HIM Study participants (from USA, Brazil and Mexico) also did not show an association between serum antibodies and reduction in subsequent infection for HPV 16 (30), although this study did not include all HIM Study participants or data for HPV genotypes 6, 11, and 18. A recent study among HIV-negative and HIV-positive men did not show protective effects against subsequent infection but the study only included MSM (men who have sex with men) subjects (31). Thus, previous studies do not provide sufficient evidence regarding the association between anti-HPV antibodies and subsequent HPV infection among all men.

Differences between men and women's antibody response to HPV infection may be due to differential sex-related immune responses, differences in assay techniques, and varying duration of serum antibody presence (30). Various studies have reported sex differences for HPV seroprevalence, with women having higher seroprevalance compared to men (93-98). HPV infection of keratinized epithelium among men may be less likely to induce an immune response, and as a result lower levels of antibodies, compared to larger mucosal surface area among women (30). Difference in assay techniques can also be a possible explanation. For example, VLP-based direct-binding ELISA assay that measures total type-specific binding IgG antibodies may yield different results compared competitive neutralization assay that binds only neutralizing epitopes in HPV viral capsid (28, 30). Assessment techniques and cut-off levels of

serum antibodies can also affect the observed association. For example, use of only high antibodies for 2 or more visits may yield different results compared to assessment of serostatus only at baseline (86, 91, 92).

Recurrent HPV infections

Among women

Few studies have examined recurrent infection or redetection of HPV infection after clearance among women. Reinfection was associated with new sexual partners, including among older women (38). Winer et al. reported that redetection of 19.4% incident infections occurred within a year (36). A study by Rodriguez et al. assessed type-specific recurrence of carcinogenic HPV types and development of subsequent cervical intraepithelial neoplasia (99). They found that 7.7% of HPV-infected women had intervening negative results, while 2.7% had definite clearance ($\geq$ 2 intervening negative results) followed by re-appearance (99). Moscicki et al. reported that 18.1% of HPV 16 infections were redetected among women who cleared an HPV 16 infection, and factors associated with risk for redetection included douching, current use of medroxyprogesterone, reporting more than one sex partner or having a new sex partner, and having a sexually transmitted infection (100).

To our knowledge, no previous study has examined the recurrence (redetection) of HPV infection after clearance of initial incident infection among men.

Concordant genital and anal HPV infections

Among women

Only a few studies have assessed concurrent genital-anal infection or sequential HPV infection starting with a genital infection followed by an anal HPV infection with the same genotype among women. Hernandez et al. assessed 1,363 women with paired anal and genital samples in a cohort of adult women in Hawaii (44). According to their results, risk of concurrent anal infection was 3-fold higher among women with cervical HPV infection as compared with women of the same age and from the same population without cervical HPV infection. They also reported a higher (26% identical and 53% partly identical) genotype-specific concordance among concurrent anal and cervical infection, possibly due to a common source of infection (44). Goodman et al. examined the genotype-concordant cervical HPV infection and subsequent anal HPV infection among 751 women in this Hawaii HPV cohort (45). They reported a Relative Risk (RR) of 20.5 (95% CI, 16.3-25.7) for acquiring a secondary anal infection after a primary cervical infection with the same HPV genotype (45). Goodman et al. also showed similar results with Hazards Ratios (HR) of 17.33 (95% CI 10.22-29.39) for any HPV type, HR of 17.95 (95% CI, 9.34–34.52) for high risk HPV and HR of 16.24 (95% CI, 6.57–40.16) for low risk HPV types (46). A cross-sectional study of 211 adult women in American Samoa by Hernandez et al, 2012 reported that 4% of women had concurrent cervical and anal HPV (101). They also found that having a cervical HPV infection was associated with anal HPV with an age-adjusted OR 3.32, 95% CI (1.10-10.00) (101). Guler et al, 2013 evaluated the prevalence of coexisting anal HPV infection and concordance of HPV types in women with cervical HPV infection (102). They reported a high prevalence (52%) of coexisting cervical and anal infections, with 20% total concordance and 58% partial concordance between the two sites (102).

To the best of our knowledge, similar associations for genotype-concordant genital and anal infection have not been examined among men. Our study will utilize the data from participants of the HIM study cohort, who agreed to provide anal samples for the study (2,103, 104). Factors associated with incidence and persistence of anal HPV infection in this cohort has been described previously (103, 104). Briefly, HPV anal HPV prevalence was 12.2% among MSW and 47.2 % among MSM subjects (103). More than 10 lifetime female sex partners and a primary sexual relationship of <1 year in duration were independently associated with anal HPV detection among MSW subjects (103). Young age, more than two male anal sex partners in the past three months, and never using a condom for anal sex in the past six months were associated with detection of any anal HPV among MSM subjects (103). The incidence rate of anal HPV 16 and 18 among MSW subjects were 0.7 and 0.9 per 1000 person-months, respectively (104). Among MSM subjects, incidence rates for HPV 16 and 18 were 4.8 and 3.8 per 1000 person-months, respectively (104). Among MSM 5.1% subjects had a persistent HPV 16 infection, while 0.6% MSW subjects had persistent infection for any genotype (104). Cigarette smoking was associated with persistent infection of any genotype among both MSM and MSW subjects (104). We will use data for MSW subjects for the analysis and examine the genotype-concordant genital and anal infection among men.

MANUSCRIPT ONE

Introduction:

Genital HPV prevalence among men exceeds 70% in some regions of the world (105), with HPV DNA detected in 29-82% of penile cancers (106, 107) and 80-100% of genital warts (108, 109). Furthermore, nearly 10,000 new cases of HPV-related oropharyngeal cancers among men are diagnosed in the U.S. each year (110). Although the antibodies produced following HPV vaccination among men provides protection against future ano-genital HPV infections and related diseases (111), it is unclear whether the antibodies produced after natural HPV infection are sufficient to protect against subsequent infection in men.

Among women, antibodies produced in response to natural HPV infection are markers of past infections and have been shown to provide partial immunity against subsequent infections and precancerous lesions (112-114); however, not all studies observed these protective effects (115-117). Differences in study findings may be due to the use of different antibody assays, serum antibody levels, and time since first exposure to HPV (118). A prospective study of HPV infection among men in Arizona did not show protective effects of circulating HPV antibodies (119). However, this study was limited by a short follow-up time, small sample size, and lack of a quantitative serum antibody assessment. An initial study of 2,187 participants in the multinational HPV Infection in Men (HIM) Study also did not show an association between serum antibodies and reduction in subsequent HPV 16 infections (118). However, this study was

limited to only one HPV type with a median duration of two years follow-up. A recent study among HIV-negative and HIV-positive men also did not show protective effects against subsequent HPV infection for multiple HPV types, but the study was restricted to men who have sex with men (MSM) (120).

In the current study, we provide the first comprehensive evaluation of incident genital HPV 6, 11, 16, 18 (any duration infection and six-month persistent infections) by baseline antibody status among the entire HIM Study cohort (n=4,123) followed for a median of 4.1 years.

Methods:

Study population

The HIM Study is an ongoing multinational study of the natural history of HPV among men in Tampa, Florida (U.S.), São Paulo (Brazil), and Cuernavaca (Mexico). Details of this study have been described previously (121). Briefly, healthy men were enrolled at each study site and followed for a median follow-up of 4.1 years, with an average interval of 6.9 months between visits. Men were eligible for the study if they: a) were 18–70 years of age; b) were residents of one of the study sites; c) had no previous diagnosis of penile or anal cancers; d) had never been diagnosed with genital or anal warts; e) had no symptoms of a sexually transmitted infection (STI) and were not receiving treatment for an STI; f) were not participating in an HPV vaccine study; g) had no history of HIV or AIDS; h) had no history of imprisonment, homelessness, or drug treatment during the past six months; and i) were willing to comply with 10 scheduled visits every six months for four years with no plans to relocate during that time. Subjects who reported never being sexually active and subjects who received HPV vaccines were excluded.

To increase access to men across a broad range of ages, sexual behaviors, and HPV risk, participants were recruited from the general population, universities, and organized healthcare systems. In Brazil, men were recruited from a facility for urogenital care (Centro de Referencia e Tratamento de Doencas Sexualmente Transmissiveis e AIDS) and through general media advertising. Men with non-sexually transmitted infections and related conditions (e.g. kidney stones, hydronephorsis, and hypertrophied bladder) were included in the study. Furthermore, partners and spouses of participants in a large cohort study of the natural history of HPV and cervical neoplasia in women conducted in São Paulo were recruited for the HIM Study. Employees and beneficiaries of the Instituto Mexicano de Seguro Social, factory employees, and permanently assigned officials of the Mexican army were recruited at the Cuernavaca, Mexico site. In the U.S., men were recruited from the University of South Florida and the greater Tampa Bay area. Flyers were distributed, and monthly educational presentations were carried out to promote the study and increase recruitment. Furthermore, mailed brochures and flyers and advertisements in local and university papers were used to recruit subjects.

All eligible participants signed an informed consent, and approval was obtained from the human subjects committees of the University of South Florida (Tampa, FL), Ludwig Institute for Cancer Research (São Paulo, Brazil), Centro de Referencia e Treinamento em Doencas Sexualmente Transmissíveis e AIDS (São Paulo, Brazil), and Instituto Nacional de Salud Publica de Mexico (Cuernavaca, Mexico). A total of 4,123 HIM Study participants were eligible for this study.

Computer assisted self-interviewing (CASI)

Each participant completed an extensive self-administered demographic and behavioral questionnaire assessing country of residence, age, race, ethnicity, marital status, education, smoking status, alcohol consumption, and a detailed sexual behavior history. The questionnaire was completed at baseline and at each six month follow-up visit. The questionnaire required approximately 20 minutes to complete and was administered using computer-assisted self-interviewing (CASI). The CASI method has been shown to be superior in reliability and consistency compared to the paper-based questionnaire method ($p < 0.05$) and is reported to be user friendly, even for computer-inexperienced subjects (122). Furthermore, the CASI method reduces errors due to faulty execution of skip instructions or confusing skip patterns and encourages more complete reporting of sensitive behaviors, such as sexual behavior history (122, 123). Other advantages of CASI include low cost, (as interviewers are not required, and there is no need to enter data into the computer separately), lower refusal rates for sensitive topics, and ease of use in multisite trials (122, 124, 125).

Baseline serum antibody testing

A 10 milliliter venous blood sample was collected at baseline to measure serum antibodies against four HPV genotypes (HPV 6, 11, 16, and 18). Serum anti-HPV antibody assessments for HPV genotypes (6, 11, 16, and 18) were carried out using a virus-like particle (VLP)-based enzyme-linked immunosorbent assay (ELISA) (126). Details of this assay have been described previously (127). Briefly, insect cells from recombinant baculoviruses expressing HPV L1 capsid proteins were used to produce HPV VLPs. Absorbance values in optical density (OD) were measured to assess seroreactivity. Serum samples from children (1-10 years old) were used

as a negative control. The cut-off point for seropositivity for each HPV genotype was selected as five SD above the mean absorbance value among the children. Positive and negative laboratory serum controls were used in each assay run for quality control purposes. Laboratory staff was blinded to participants' HPV DNA status.

External genital HPV DNA samples

Three prewetted Dacron swabs were used to collect genital cell specimens from the coronal sulcus/glans penis, penile shaft, and scrotum and were later combined to form a single sample (121, 128). All specimens were stored at -80°C until genotyping was conducted. The QIAamp DNA Blood Mini Kit (QIAGEN) was used to extract DNA from genital cell specimens. Roche Linear Array kits were used for PCR and HPV DNA genotyping to detect 37 different types of HPV (129). The presence of human β-globin was used to assess specimen adequacy with an overall β-globin positivity of >98%.

Statistical analysis

Demographic characteristics were compared between seronegative and seropositive men at baseline using Chi-square tests with Monte Carlo estimation of exact p-values. Only men who were DNA-negative for the respective HPV type at the baseline visit were included in analyses. Separate analyses were carried out for each of the four HPV genotypes (HPV 6, 11, 16 and 18) by duration of infection (any duration for incident infections and $\geq$6 months duration for persistent infection).

Incidence proportions (all incident HPV 6, 11, 16, and 18 infections regardless of duration) and six-month persistent incidence proportions were calculated at each six-month interval among subjects who had one or more follow-up visits after baseline. Follow-up times were calculated

based on visit dates and participants who were late for their visit were included in the appropriate time interval for the analysis. To estimate incidence proportions, the first detection of HPV DNA at a follow-up visit was defined as an incident HPV infection (numerator). The number of subjects who tested negative for HPV DNA at the beginning of each study interval was defined as the at-risk population (denominator). For six-month persistent infections, a persistent infection was defined as HPV DNA detection at two or more consecutive visits. Persistent infections also included subjects with an intervening negative result (n= 20 for HPV 6, n=5 for HPV 11, n=22 for HPV 16 and n= 11 for HPV 18). Participants who were DNA- negative for the given HPV type (at risk of acquiring a new infection) at the beginning of each study interval were defined as at-risk subjects for six-month persistent infection.

Kaplan-Meier (KM) curves were constructed for incident and six-month persistent infections for each of the four HPV genotypes (6, 11, 16 and 18). Cumulative incidence for each genotype was compared between seropositive and seronegative subjects, with the log-rank test used to determine significant differences by serum antibody status. Cox proportional hazard models were used to calculate crude and adjusted hazard ratios (aHR) and 95% confidence intervals (CI). A list of candidate variables was created based on descriptive analyses, previous literature, and assessment of confounding by each variable. Variables were evaluated in both backward and forward stepwise models. Final models included variables based on best-fit approach based on the lowest Akaike information criterion (AIC) values. As sexual behavior prior to acquisition of incident infections strongly influences HPV acquisition, we included the following sexual behavior variables as time-varying covariates in final adjusted Cox models: lifetime number of female sexual partners, number of new female sexual partners in the past 6-12 months, frequency of sexual intercourse with female partners in the past 6-12 months, lifetime

number of male sexual partners, and number of new male sexual partners in the past 6-12 months. Due to the differences in HPV seroprevalence by sexual orientation, stratified analyses were also conducted. To evaluate whether antibody levels were associated with subsequent detection of incident infections, we categorized serum antibody levels into the highest tertile and lowest two tertiles and compared these two groups to seronegative men (113, 120).

Results:

Serum antibody and HPV genotyping data were available for 4,103 subjects at baseline. We excluded 250 (HPV 6), 55 (HPV 11), 311(HPV 16) and 90 (HPV 18) subjects who were DNA-positive at the baseline visit for the respective HPV type. Therefore, baseline seroprevalence analyses included 3,851 (HPV 6), 4,046 (HPV 11), 3,790 (HPV 16), and 4,011 (HPV 18) subjects who were DNA-negative for the specific HPV genotype.

Overall, baseline HPV6, 11, 16, and 18 seroprevalence was 8.1%, 13.9%, 12.4%, and 10.8%, respectively. Significant differences were observed between seropositive and seronegative men for various covariates (Table 2.1). Seroprevalence increased with increasing age, with the highest seroprevalence for HPV 6 (9.4%), 11 (16.2%), and 16 (16.4%) observed among men ages 31-44, and the highest HPV 18 seroprevalence (17.9%) among men ages 45-73. Brazil had the highest seroprevalence for HPV 6 (10.8%), 16 (16.9%), and 18 (13.0%), while Mexico had the highest seroprevalence for HPV 11 (18.8%). Men who have sex with men (MSM) had the highest seroprevalence for all four genotypes (HPV 6: 23.2%, HPV 11: 27.6%, HPV 16: 28.6%, and HPV 18: 29.3%). Seroprevalence was highest among men reporting ≥10 lifetime male sexual partners (30.0%, 37.8%, 39.0%, and 40.4% for HPV 6, 11, 16, and 18 respectively).

For HPV 6, 11, 16, and 18, the total numbers of incident infections were 319 (10.3%), 111 (3.5%), 362 (12.4%), and 200 (6.2%) among seronegative men, and 30 (10.6%), 17 (3.3%), 70 (16.7%), and 22 (5.6%) among seropositive individuals, respectively. Cumulative incidence among seropositive individuals was higher after 18 months for HPV 16 (p-value= 0.007, Figure 2.1a, table 2.2a). For HPV 11 and 18, incidence proportions were generally lower among seropositive individuals until the 13-18 month interval, followed by similar proportions (HPV 11 and 18) after 24 months. KM curves indicated a similar pattern with overall non-significant p-values (p = 0.76 for HPV 11 and p = 0.69 for HPV 18) for the association between serostatus and HPV acquisition. For HPV 6, cumulative incidences among seropositive and seronegative subjects was similar (p-value= 0.728).

The total numbers of incident six-month persistent infections were 100 (3.5%), 37 (1.3%), 120 (4.5%), and 74 (2.5%) among seronegative men and 7 (2.8%), 2 (0.4%), 21 (5.5%), and 2 (0.6%) among seropositive individuals for HPV 6, 11, 16 and 18, respectively. Six month persistent HPV 16 infections did not differ significantly between seronegative and seropositive individuals (Figure 2.1b, Table 2.2b). The overall number of six-month persistent HPV 11 and 18 infections observed was low (two infections for each type over 4.1 year follow-up period). Six-month persistent infections were not observed prior to month 31 among HPV 11seropositive individuals and prior to month 19 for HPV18 seropositive individuals. Significantly lower incidence of six-month persistent HPV 18 infections was observed among seropositive individuals (p=0.02).

Risk of an incident HPV 16 infection was significantly higher among seropositive compared to seronegative men (aHR 1.37, 95% CI 1.01, 1.86) (Table 2.3). A similar non-significant pattern was observed for risk of six-month persistent HPV 16 infections (aHR 1.26,

95% CI 0.79, 2.01). Serostatus was not associated with risk of HPV 6, 11, and 18 incident infections. However, risk of persistent HPV 18 infection was significantly lower among seropositive men in unadjusted analyses (crude HR 0.22, 95% CI 0.06-0.91), but failed to reach significance in the adjusted model (aHR 0.19, 95% CI 0.03, 1.37). In separate analyses adjusting for demographic and sexual behavior variables one at a time, similar results to those shown in Table 3.3 were observed (Appendix Table 2.1).

Table 3.4 shows the risk of incident and six-month persistent HPV 16 and 18 infections by serostatus and sexual orientation. No significant associations were observed likely due to the relatively small sample sizes in each stratum. No evidence of effect modification was observed for HPV 18. However, serostatus was non-significantly associated with a decreased risk among MSM and increased risk among heterosexual men. Similarly, no significant associations were observed for HPV 6 and 11 (Appendix Table 2.2). In analyses by serum antibody levels, high levels (highest tertile) of antibody were not significantly associated with reduced risk of subsequent genital HPV infection (Appendix Table 2.3). In analyses comparing the lower two tertile groups to the seronegative group, the incidence rate ratio for HPV 16 was 1.40 (945% CI, 1.01-1.89) and for persistent HPV 18 was 0.16 (95% CI 0.004-0.93).These results are comparable to the results discussed previously.

To address the issue of small sample size and to increase power, we conducted analyses by combining seropositive individuals for any of the four HPV types and compared them to seronegative individuals for all four HPV types. These analyses did not indicate significantly higher HR for subsequent HPV 16 incident infection, but for persistent HPV 18 infection a significant protective effect was observed in adjusted models (aHR 0.42, 95% CI 0.19, 0.93) (Table 2.5).

Discussion:

In this study of men, baseline seropositive status was not associated with a reduction in subsequent incident or six-month persistent HPV 6, 11, and 16 infections. In fact, HPV 16 seropositive men had a higher HPV 16 incident infection rate compared to HPV 16 seronegative men. A possible protective effect was observed for HPV 18 persistent infections among seropositive individuals, but the small number of infections requires a cautious interpretation of these results. In general, natural immunity after HPV infection was not associated with a reduced risk for subsequent HPV infections among men.

This is the first study to report a significantly higher risk of incident genital HPV 16 infections among seropositive men. In the few studies that have assessed the association of antibodies generated following natural HPV infection and protection against subsequent genital HPV infections in men, results have either been inconclusive or have not shown an association (118, 119, 112). The current study is an extended analysis of previous work from our group (118), in which we showed a non-significant increased risk for incident (aHR 1.22, 95% CI 0.83, 1.79) and persistent HPV 16 genital infections (aHR 1.05, 95% CI 0.51, 2.16).

The higher HPV 16 incidence among seropositive men was not observed consistently over the entire follow-up period. Similar or lower genital HPV 16 incidence was observed among seropositive and seronegative men up to the month 18 follow-up visit. Similar initial protective effects against HPV 16 and other HPV genotypes have been documented among women, followed by gradual waning of naturally induced IgG antibody levels over time (130-136). High-risk sexual behavior among seropositive men, compared to seronegative men, may have led to higher rates of incident infections among them. However, if this was the case, higher incidence

among seropositive men should have been observed for other HPV types as well, and not just for HPV 16. Although we adjusted for sexual behavior variables as time-varying covariates, there is still a possibility that residual confounding occurred. There is also a possibility of not capturing precise sexual behavior related risk due to error in reporting sexual behavior in the self-reported questionnaire. Higher risk of incident and persistent infections among HPV 16 seropositive individuals may also indicate possible reactivation of prior latent infection (137).

In this study, incident and persistent HPV 11 and 18 infections were generally lower among seropositive subjects. Lower incidence (especially for persistent HPV 18) may be suggestive of a transient protective effect, which gradually wanes after 18 months, as the KM analyses indicated. However, due to the small number of HPV 18 events, this association failed to reach statistical significance. Additional studies are needed to understand the role of anti-HPV 18 antibodies among men.

Factors including differential immune response by gender, differences in assay techniques, and different durations of antibody levels have been suggested as possible explanations for the difference in antibody response observed among men and women (118, 131, 138, 139). Numerous studies have indicated a higher seroprevalence of both oncogenic and non-oncogenic HPV types among women compared with men (140-146). A lower level of antibody response following infection of keratinized epithelium among heterosexual men compared to mucosal epithelial infection in heterosexual women may be a possible explanation for this gender difference (118). Inclusion of non-neutralizing antibodies in our study may have led to overestimation of overall serum antibody levels and may have biased the results toward the null. Serum antibody cut-off levels may also affect the observed association. We therefore carried out

analyses using the highest versus the lowest two tertiles of serum antibodies, and the results were not substantially different.

To the best of our knowledge, our study is the first to assess the effect of serum antibody status for all four major vaccine type HPVs in a large, multicenter cohort of men with a long-term follow-up. Despite these strengths, some limitations may have influenced the results. Serum antibody levels were determined at a single baseline visit in our study. Men with prior exposure may have been misclassified as seronegative due to lower seroconversion rates, waning antibody response, and time-lag between exposure and antibody response. This misclassification is likely to be non-differential and may have attenuated findings toward the null. Misclassification of HPV infection is also a possibility, although we utilized robust methods for DNA detection that are well-established (121). Furthermore, single baseline visit DNA negative subjects were assumed to be the at risk population and were considered eligible for the study. We ran a separate analysis using more stringent criteria of requiring two negative visits (baseline and first six months visit) to qualify for entry into the analyses. Use of this stringent criterion also did not alter study results.

We did not have information about infections that may have occurred prior to the study start. These infections may not have cleared but instead remained latent at low copy numbers (137). Latency for HPV infection has been described in animal models and recently, reactivation of latent infection has been demonstrated among immuno-compromised individuals (137). Assessment of previous infections is challenging, especially among men who have been sexually active for a long period of time. Furthermore, current HPV DNA detection methods may not be sensitive enough to detect low viral load of latent infection or reactivations that last for a short

time period (118). Future studies are needed to explore the effect of latent HPV infections and their reactivation among healthy individuals.

In conclusion, among men participating in the HIM study, baseline seropositivity after natural infection with HPV 6, 11 and 16 was not associated with protection against subsequent type-specific genital infection, with a possible protective effect against persistent HPV 18 infections. For HPV 16, a significantly higher risk of incident infection was observed among seropositive men. The effect of anti-HPV antibodies may be influenced by duration of the antibody response, time period between initial and subsequent genital infection, and overall antibody levels. These results highlight the importance of HPV prevention measures, such as vaccination, which are proven to protect against subsequent HPV infections and related genital diseases.

Table 2.1- Demographic characteristics of seronegative and HPV 6, 11, 16 and 18 seropositive HIM Study participants

	HPV 6		*HPV 11*		*HPV 16*		*HPV 18*	
Characteristics	***Seronegative***	***Seropositive***	***Seronegative***	***Seropositive***	***Seronegative***	***Seropositive***	***Seronegative***	***Seropositive***
Country								
United States	1216 (96.4)	46 (3.6)	1249 (94.0)	80 (6.0)	1086 (88.6)	140 (11.4)	1209 (93.2)	88 (6.8)
Brazil	1173 (89.2)	142 (10.8)	1155 (83.1)	235 (16.9)	1047 (81.7)	234 (18.3)	1199 (87.0)	179 (13.0)
Mexico	1149 (90.2)	125 (9.8)	1077 (81.2)	250 (18.8)	1176 (91.7)	107 (8.3)	1170 (87.6)	166 (12.4)
p value[a]		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**
Age								
18-30	1723 (93.1)	127 (6.9)	1740 (88.2)	232 (11.8)	1655 (90.9)	165 (9.1)	1786 (91.8)	159 (8.2)
31-44	1354 (90.6)	140 (9.4)	1296 (83.8)	250 (16.2)	1225 (83.6)	241 (16.4)	1366 (88.3)	181 (11.7)
45-73	461 (90.9)	46 (9.1)	445 (84.3)	83 (15.7)	429 (85.1)	75 (14.9)	426 (82.1)	93 (17.9)
p value[a]		**0.0209**		**<0.0001**		**<0.0001**		**<0.0001**
Marital status								
Single	1583 (92.6)	127 (7.4)	1606 (88.2)	214 (11.8)	1475 (87.9)	203 (12.1)	1612 (90.3)	174 (9.7)
Married/ Cohabitating	1628 (91.3)	156 (8.7)	1555 (83.9)	298 (16.1)	1544 (87.6)	218 (12.4)	1648 (88.8)	208 (11.2)
Divorced/Separated	307 (91.6)	28 (8.4)	300 (85.7)	50 (14.3)	272 (82.4)	58 (17.6)	296 (85.3)	51 (14.7)
p value[a]		0.3578		**0.0016**		**0.0217**		**0.0209**
Race								
Whites	1590 (93.3)	114 (6.7)	1587 (87.7)	223 (12.3)	1419 (85.2)	246 (14.8)	1606 (90.5)	169 (9.5)
African-Americans	547 (91)	54 (9.0)	561 (88.6)	72 (11.4)	486 (83.5)	96 (16.5)	547 (87.4)	79 (12.6)
Asian/Pacific Islander	105 (98.1)	2 (1.9)	105 (93.8)	7 (6.3)	100 (92.6)	8 (7.4)	101 (93.5)	7 (6.5)
Mixed race/Others [b]	1236 (89.8)	141 (10.2)	1171 (81.9)	258 (18.1)	1254 (90.9)	125 (9.1)	1265 (87.9)	174 (12.1)
p value[a]		**<0.0001**		**<0.0001**		**<0.0001**		**0.0213**

Table 2.1(Continued)

	HPV 6		*HPV 11*		*HPV 16*		*HPV 18*	
Characteristics	***Seronegative***	***Seropositive***	***Seronegative***	***Seropositive***	***Seronegative***	***Seropositive***	***Seronegative***	***Seropositive***
Education								
≤ 12 years	1689 (89.9)	189 (10.1)	1653 (83.8)	319 (16.2)	1622 (86.9)	244 (13.1)	1750 (88.8)	221 (11.2)
13-15 years	931 (95.0)	49 (5.0)	933 (91.3)	89 (8.7)	854 (89.9)	96 (10.1)	917 (90.9)	92 (9.1)
≥16 years	895 (92.5)	73 (7.5)	875 (85.3)	151 (14.7)	813 (85.5)	138 (14.5)	888 (88.4)	117 (11.6)
p value[a]		**<0.0001**		**<0.0001**		**0.0113**		0.1369
Alcohol use								
Non-drinkers	834 (89.8)	95 (10.2)	822 (85.0)	145 (15.0)	803 (86.9)	121 (13.1)	846 (87.9)	117 (12.1)
<0.5 drinks/day	1173 (91.9)	103 (8.1)	1133 (85.1)	199 (14.9)	1110 (88.3)	147 (11.7)	1169 (88.3)	155 (11.7)
0.5-2 drinks/day	817 (93.1)	61 (6.9)	813 (87.4)	117 (12.6)	760 (88.1)	103 (11.9)	832 (91.3)	79 (8.7)
>2 drinks/day	563 (92.9)	43 (7.1)	566 (87.3)	82 (12.7)	506 (85.6)	85 (14.4)	574 (89.4)	68 (10.6)
p value[a]		**0.0469**		0.2322		0.3649		0.0649
Cigarette smoking								
Never	2059 (92.9)	158 (7.1)	2020 (87)	302 (13.0)	1912 (87.5)	272 (12.5)	2072 (90.2)	226 (9.8)
Former smoker	644 (91)	64 (9.0)	636 (85)	112 (15.0)	612 (86.9)	92 (13.1)	646 (86.8)	98 (13.2)
Current smoker	811 (90.3)	87 (9.7)	802 (84.4)	148 (15.6)	760 (86.8)	116 (13.2)	832 (88.5)	108 (11.5)
p value[a]		**0.0244**		0.1118		0.8047		**0.0302**
Circumcision								
No	2152 (90.2)	233 (9.8)	2078 (83.2)	421 (16.8)	2045 (86.8)	310 (13.2)	2195 (88.0)	299 (12.0)
Yes	1386 (94.5)	80 (5.5)	1403 (90.7)	144 (9.3)	1264 (88.1)	171 (11.9)	1383 (91.2)	134 (8.8)
p value[a]		**<0.0001**		**<0.0001**		0.2636		**0.0016**
Sexual orientation								
MSW	2664 (93.5)	186 (6.5)	2640 (88)	360 (12.0)	2504 (89.3)	300 (10.7)	2704 (91)	267 (9.0)
MSM	86 (76.8)	26 (23.2)	84 (72.4)	32 (27.6)	80 (71.4)	32 (28.6)	82 (70.7)	34 (29.3)
MSWM	325 (83.8)	63 (16.2)	312 (75.5)	101 (24.5)	291 (76.6)	89 (23.4)	328 (80.2)	81 (19.8)
p value[a]		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**

Table 2.1(Continued)

	HPV 6		*HPV 11*		*HPV 16*		*HPV 18*	
Characteristics	***Seronegative***	***Seropositive***	***Seronegative***	***Seropositive***	***Seronegative***	***Seropositive***	***Seronegative***	***Seropositive***
Lifetime female sexual partners								
0-1 partners	764 (92.7)	60 (7.3)	487 (87.4)	70 (12.6)	483 (89.4)	57 (10.6)	518 (92.3)	43 (7.7)
2-9 partners	1171 (92.7)	92 (7.3)	1393 (86.6)	216 (13.4)	1362 (89.4)	162 (10.6)	1449 (90.8)	147 (9.2)
10-49 partners	1082 (92.6)	87 (7.4)	1080 (86.2)	173 (13.8)	987 (86.6)	153 (13.4)	1096 (88.5)	142 (11.5)
≥50 partners	193 (88.9)	24 (11.1)	201 (87)	30 (13.0)	171 (80.7)	41 (19.3)	192 (85.3)	33 (14.7)
p value[a]		0.2589		0.9058		**0.0010**		**0.0041**
New female partners in past 3-6 months								
None	2020 (92.3)	169 (7.7)	1982 (86.6)	306 (13.4)	1898 (87.7)	265 (12.3)	2027 (89.5)	238 (10.5)
1 partner	809 (93.1)	60 (6.9)	804 (86.7)	123 (13.3)	768 (89.6)	89 (10.4)	835 (90.6)	87 (9.4)
≥2 partners	389 (93.3)	28 (6.7)	392 (88.5)	51 (11.5)	343 (85.3)	59 (14.7)	395 (91.0)	39 (9.0)
p value[a]		0.6271		0.5583		0.0838		0.4886
Lifetime male sexual partners								
None	2765 (93.6)	188 (6.4)	2745 (88.2)	366 (11.8)	2602 (89.6)	302 (10.4)	2808 (91.2)	270 (8.8)
1-9 partners	306 (87.4)	44 (12.6)	299 (80.2)	74 (19.8)	282 (81.5)	64 (18.5)	317 (85.9)	52 (14.1)
≥10 partners	105 (70.0)	45 (30.0)	97 (62.2)	59 (37.8)	89 (61)	57 (39.0)	93 (59.6)	63 (40.4)
p value[a]		**<0.0001**		**<0.00**		**<0.0001**		**<0.0001**
New male partners in past 3 months								
None	3054 (92.7)	239 (7.3)	3015 (87)	450 (13)	2865 (88.6)	370 (11.4)	3100 (90.4)	329 (9.6)
1 partner	57 (77)	17 (23.0)	59 (72)	23 (28.0)	51 (67.1)	25 (32.9)	60 (72.3)	23 (27.7)
≥2 partners	64 (72.7)	24 (27.3)	66 (70.2)	28 (29.8)	57 (66.3)	29 (33.7)	56 (60.9)	36 (39.1)
p value[a]		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**

Footnotes:

Abbreviation: HPV, human papillomavirus; MSM, men who have sex with men; MSW, men who have sex with women; MSWM, men who have sex with women and men

a - p-values for categorical variables were calculated using the chi-square test with Monte Carlo estimation of p-values, $\alpha = 0.05$.

b - Includes mixed race, American Indians, Alaska natives, Native Hawaiians or other races

Table 2.2a –HPV6, 11, 16 and 18 incidence proportions during 4 years follow-up in HIM Study

Time of DNA detection	HPV 6			HPV 11			HPV 16			HPV 18		
	At risk[a]	N	IP (95% CI) [b]	At risk[a]	N	IP (95% CI) b	At risk[a]	N	IP (95% CI) b	At risk[a]	N	IP (95% CI) b
0-6 months												
Seronegative	2371	47	1.98 (1.42, 2.54)	2384	13	0.55 (0.25, 0.84)	2227	45	2.02 (1.44, 2.61)	2454	26	1.06 (0.65, 1.46)
Seropositive	214	5	2.34 (0.31, 4.36)	393	0	0.00 (NA)	316	7	2.22 (0.59, 3.84)	298	0	0.00 (NA)
7-12 months												
Seronegative	2615	53	2.03 (1.49, 2.57)	2681	22	0.82 (0.48, 1.16)	2443	52	2.13 (1.56, 2.70)	2717	32	1.18 (0.77, 1.58)
Seropositive	233	6	2.58 (0.54, 4.61)	437	2	0.46 (0.18, 1.09)	364	5	1.37 (0.18, 2.57)	333	2	0.60 (0.23, 1.43)
13-18 months[c]												
Seronegative	2539	58	2.28 (1.70, 2.87)	2607	14	0.54 (0.26,0.82)	2382	55	2.31 (1.71, 2.91)	2673	33	1.23 (0.82, 1.65)
Seropositive	228	4	1.75 (0.05, 3.46)	434	1	0.23 (0.22, 0.68)	345	5	1.45 (0.19, 2.71)	322	5	1.55 (0.20, 2.90)
19-24 months												
Seronegative	2436	29	1.19 (0.76, 1.62)	2528	15	0.59 (0.29, 0.89)	2249	60	2.67 (2.00, 3.33)	2554	23	0.90 (0.53, 1.27)
Seropositive	201	4	1.99 (0.06, 3.92)	406	3	0.74 (0.09, 1.57)	337	17	5.04 (2.71, 7.38)	319	4	1.25 (0.03, 2.48)
25-30 months												
Seronegative	2258	33	1.46 (0.97, 1.96)	2376	10	0.42 (0.16, 0.68)	2089	28	1.34 (0.85, 1.83)	2417	22	0.91 (0.53, 1.29)
Seropositive	194	3	1.55 (0.19, 3.28)	401	2	0.50 (0.19, 1.19)	292	9	3.08 (1.10, 5.06)	279	0	0.00 (NA)
31-36 months												
Seronegative	2215	32	1.44 (0.95, 1.94)	2329	12	0.52 (0.22, 0.81)	2031	44	2.17 (1.53, 2.80)	2351	18	0.77 (0.41, 1.12)
Seropositive	205	4	1.95 (0.06, 3.84)	392	2	0.51 (0.20, 1.22)	297	10	3.37 (1.32, 5.42)	303	3	0.99 (0.12, 2.10)
37-42 months												
Seronegative	1938	24	1.24 (0.75, 1.73)	2058	18	0.87 (0.47, 1.28)	1741	32	1.84 (1.21, 2.47)	2072	16	0.77 (0.40, 1.15)
Seropositive	157	2	1.27 (0.48, 3.03)	347	7	2.02 (0.54, 3.50)	242	10	4.13 (1.62, 6.64)	275	4	1.45 (0.04, 2.87)
43-48 months												
Seronegative	1716	31	1.81 (1.18, 2.44)	1863	4	0.21 (0.00, 0.42)	1581	32	2.02 (1.33, 2.72)	1846	16	0.87 (0.44 , 1.29)
Seropositive	158	1	0.63 (0.60, 1.87)	321	0	0.00 (NA)	245	6	2.45 (0.51, 4.38)	248	3	1.21 (0.15, 2.57)
>48 months												
Seronegative	559	12	2.15 (0.95, 3.35)	623	3	0.48 (1.97, 2.32)	526	14	2.66 (1.29, 4.04)	628	14	2.23 (1.07, 3.38)
Seropositive	37	1	2.70 (2.52, 7.93)	93	0	0.00 (NA)	55	1	1.82 (1.70, 4.37)	64	1	1.56 (1.48, 4.60)

Footnotes –

Abbreviations: HPV-human papilloma virus; IP-Incidence proportion, CI-confidence interval; NA-Not Applicable, Sero- = Seronegative, Sero+ = Seropositive

a - No. at risk was defined as number of men followed till the stated duration, who were HPV DNA negative for particular HPV type at the beginning of time interval

b - Percentage was defined as the number of incident infection over the number of participants at risk that occurred at each time interval

c - Participants who were late (1st follow-up visit after six months), were included in successive time interval based on the visit date, which led to higher numbers for second follow-up period.

Table 2.2b – HPV 6, 11, 16 and 18 incident persistent infections during 4 years follow-up in HIM Study

Time of DNA detection	HPV 6			HPV 11			HPV 16			HPV 18		
	At risk[a]	N	IP (95% CI) [b]	At risk[a]	N	IP (95% CI) [b]	At risk[a]	N	IP (95% CI) [b]	At risk[a]	N	IP (95% CI) [b]
7-12 months												
Seronegative	2012	11	0.55 (0.22, 0.87)	2035	7	0.34 (0.09, 0.60)	1885	15	0.80 (0.39, 1.20)	2079	8	0.38 (0.12, 0.65)
Seropositive	174	1	0.57 (0.55, 1.70)	332	0	0.00 (NA)	277	2	0.72 (0.28, 1.72)	256	0	0.00 (NA)
13-18 months[c]												
Seronegative	2539	22	0.87 (0.51, 1.23)	2571	4	0.16 (0.00, 0.31)	2389	26	1.09 (0.67, 1.50)	2657	16	0.60 (0.31, 0.90)
Seropositive	233	2	0.86 (0.33, 2.04)	431	0	0.00 (NA)	344	2	0.58 (0.22, 1.38)	315	0	0.00 (NA)
19-24 months												
Seronegative	2464	17	0.69 (0.36, 1.02)	2530	8	0.32 (0.10, 0.53)	2302	28	1.22 (0.77, 1.66)	2561	18	0.70 (0.38, 1.03)
Seropositive	202	1	0.50 (0.47, 1.46)	398	0	0.00 (NA)	337	4	1.19 (0.03, 2.34)	319	1	0.31 (0.30, 0.93)
25-30 months												
Seronegative	2283	16	0.70 (0.36, 1.04)	2380	5	0.21 (0.03, 0.39)	2120	13	0.61 (0.28, 0.95)	2435	10	0.41 (0.16, 0.66)
Seropositive	199	2	1.01 (0.38, 2.39)	402	0	0.00 (NA)	305	3	0.98 (0.12, 2.09)	283	0	0.00 (NA)
31-36 months												
Seronegative	2248	12	0.53 (0.23, 0.84)	2340	5	0.21 (0.03, 0.40)	2061	13	0.63 (0.29, 0.97)	2371	6	0.25 (0.05, 0.46)
Seropositive	207	1	0.48 (0.46, 1.43)	395	1	0.25 (0.24, 0.75)	311	3	0.96 (0.12, 2.05)	303	0	0.00 (NA)
37-42 months												
Seronegative	1956	6	0.31 (0.06, 0.55)	2062	3	0.15 (0.02, 0.31)	1773	15	0.85 (0.42, 1.27)	2082	7	0.34 (0.09, 0.58)
Seropositive	160	0	0.00 (NA)	353	1	0.28 (0.27, 0.84)	252	4	1.59 (0.04, 3.13)	275	0	0.00 (NA)
43-48 months												
Seronegative	1737	12	0.69 (0.30, 1.08)	1869	4	0.21 (0.00, 0.42)	1600	9	0.56 (0.20, 0.93)	1857	6	0.32 (0.06, 0.58)
Seropositive	159	0	0.00 (NA)	321	0	0.00 (NA)	248	2	0.81 (0.31, 1.92)	252	0	0.00 (NA)
>48 months												
Seronegative	569	4	0.70 (0.02, 1.39)	627	1	0.16 (0.15, 0.47)	536	1	0.19 (0.18, 0.55)	634	3	0.47 (0.06, 1.01)
Seropositive	37	0	0.00 (NA)	95	0	0.00 (NA)	60	1	1.67 (1.57, 4.91)	65	1	1.54 (1.45, 4.53)

Footnotes –

Abbreviations: HPV, human papilloma virus; CI, confidence interval; NA, Not Applicable

a - No. at risk was defined as number of men followed till the stated duration, who were HPV DNA negative or positive for particular HPV type at the beginning of each time interval

b - Percentage was defined as the number of incident infection over the number of participants at risk that occurred at each time interval

c - Participants who were late (1st follow-up visit after six months), were included in successive time interval based on the visit date, which led to higher numbers for second follow-up period.

Table 2.3 - Crude and adjusted hazard ratios (HR) according to sero-status for incident HPV 6, 11, 16 and 18 infections among HIM Study participants

	No. of men	No. of infections	Crude HR and 95% CI	Adjusted HR and 95% CI
Incident infection				
HPV 6				
Seronegative	3105	319	1.00	1.00
Seropositive	283	30	1.08 (0.74, 1.56)	0.91 (0.56, 1.48)[a]
HPV 11				
Seronegative	3132	111	1.00	1.00
Seropositive	513	17	0.92 (0.55, 1.54)	0.80 (0.44, 1.45)[b]
HPV 16				
Seronegative	2912	362	1.00	1.00
Seropositive	420	70	**1.40 (1.08, 1.81)**	**1.37 (1.01, 1.86)[c]**
HPV 18				
Seronegative	3202	200	1.00	1.00
Seropositive	391	22	0.92 (0.59, 1.43)	0.90 (0.531, 1.57)[d]
6 month persistent infection				
HPV 6				
Seronegative	3105	100	1.00	1.00
Seropositive	283	7	0.80 (0.37, 1.73)	0.98 (0.39, 2.46)[e]
HPV 11				
Seronegative	3132	37	1.00	1.00
Seropositive	513	2	0.32 (0.08, 1.34)	0.31 (0.04, 2.30)[f]
HPV 16				
Seronegative	2912	120	1.00	1.00
Seropositive	420	21	1.26 (0.79, 2.01)	1.39 (0.80, 2.41)[g]
HPV 18				
Seronegative	3202	74	1.00	1.00
Seropositive	391	2	**0.22 (0.06, 0.91)**	0.19(0.03 1.37)[h]

Footnotes: HPV- Human Papillomavirus, HR- Hazard ratios

a - Adjusted for marital status, alcohol use, lifetime male sexual partners (time varying), and new female partners in past 6-12 months (time varying).

b - Adjusted for marital status, race, lifetime female sexual partners (time varying), and new male partners in past 6-12 months (time varying).

c - Adjusted for age, marital status, lifetime female sexual partners (time varying), lifetime male sexual partners (time varying), and new female partners in past 6-12 months (time varying).

d - Adjusted for country, alcohol use, lifetime male sexual partners (time varying), and new female partners in past 6-12 months (time varying).

e - Adjusted for age, alcohol use, new female partners in past 6-12 months (time varying) and frequency of sexual intercourse with female partners in past 6-12 months.

f - Adjusted for age, alcohol use, new female partners in past 6-12 months (time varying) and new male partners in past 6-12 months (time varying)

g - Adjusted for age, country, lifetime male sexual partners (time varying) and new female partners in past 6-12 months (time varying)

h - Adjusted for marital status, alcohol use, lifetime male sexual partners (time varying), and new female partners in past 6-12 months (time varying).

Table 2.4 - Crude and adjusted hazard ratios (HR) stratified by sexual orientation for incident HPV 16 and 18 infections among HIM Study participants

	HPV 16				HPV 18			
	At risk	**N**	**Crude HR**	**Adjusted HR**	**At risk**	**N**	**Crude HR**	**Adjusted HR**
Incident infections								
MSW								
Seronegative	2204	269	1.0	1.0	2419	138	1.0	1.0
Seropositive	261	42	1.37 (0.99, 1.90)	1.31(0.90,1.93)[a]	242	11	0.82 (0.45, 1.52)	1.01 (0.51, 2.01)[d]
MSWM								
Seronegative	268	47	1.0	1.0	305	27	1.0	1.0
Seropositive	82	14	1.06 (0.58, 1.93)	0.88 (0.45, 1.73)[b]	73	7	1.12 (0.49, 2.56)	1.02 (0.41, 2.58)[e]
MSM								
Seronegative	75	12	1.0	1.0	78	10	1.0	1.0
Seropositive	27	6	1.33 (0.50, 3.55)	1.61 (0.58, 4.53)[c]	31	2	0.44 (0.09, 2.03)	0.98 (0.17, 5.67)[c]
Six month persistent infections								
MSW								
Seronegative	2204	93	1.0	1.0	2419	53	1.0	1.0
Seropositive	261	15	1.42 (0.82, 2.44)	1.46 (0.79, 2.73)[f]	242	1	0.19 (0.03, 1.41)	0.25 (0.03,1.84)[i]
MSWM								
Seronegative	268	13	1.0	1.0	305	9	1.0	1.0
Seropositive	82	3	0.83 (0.24, 2.93)	0.85 (0.24, 3.00)[g]	73	1	0.49 (0.06, 3.84)	0.51 (0.06, 4.03)[h]
MSM								
Seronegative	75	5	1.0	1.0	78	1	1.0	1.0
Seropositive	27	1	0.54 (0.06, 4.60)	0.58 (0.07, 4.97)[h]	31	0	NE	NE

Footnotes:

HPV- Human Papillomavirus, HR- Hazard ratios, MSW- Men who have sex with women, MSWM- Men who have sex with women and men, MSM- Men

who have sex with men

a-Adjusted for race, alcohol use, lifetime female partners (time varying) and new female partners in past 6-12 months(time varying)

b-Adjusted for marital status, alcohol use, new female partners in past 6-12 months(time varying) and new male partners in past 6-12 months(time varying)

c - Adjusted for new male partners in past 6-12 months(time varying)

d- Adjusted for marital status, alcohol use, new female partners in past 6-12 months(time varying) and frequency of sexual intercourses in past 6-12 months(time varying)

e- Adjusted for age, new male partners in past 6-12 months(time varying) and frequency of sexual intercourse with female partners in past 6-12 months(time varying)

f-Adjusted for age, country, and new female partners in past 6-12 months(time varying)

g- Adjusted for country

h- Adjusted for age

i- Adjusted for marital status, lifetime female partners(time varying) and new female partners in past 6-12 months(time varying)

Table 2.5: Crude and adjusted hazard ratios (HR) according to seropositive for any four HPV types (HPV 6, 11, 16 and 18) compared to seronegative for all four types infections among men in the HIM study

	No. of men	No. of infections	Crude HR and 95% CI	Adjusted HR and 95% CI
Incident infection				
HPV 6				
Seronegative[a]	2663	240	1.00	1.00
Seropositive[b]	1082	109	1.12 (0.90, 1.41)	1.08(0.81, 1.43)[c]
HPV 11				
Seronegative[a]	2858	88	1.00	1.00
Seropositive[b]	1164	40	1.12 (0.77, 1.63)	0.89 (0.55, 1.42)[d]
HPV 16				
Seronegative[a]	2609	307	1.00	1.00
Seropositive[b]	1062	125	1.01 (0.82, 1.24)	1.08 (1.84, 1.39)[e]
HPV 18				
Seronegative[a]	2803	157	1.00	1.00
Seropositive[b]	1158	65	1.00 (0.75, 1.34)	1.09 (0.77, 1.54)[f]
6 month persistent infection				
HPV 6				
Seronegative[a]	2663	83	1.00	1.00
Seropositive[b]	1082	24	0.72 (0.46, 1.13)	0.82 (0.48, 1.40)[g]
HPV 11				
Seronegative[a]	2858	30	1.00	1.00
Seropositive[b]	1164	9	0.73 (0.35, 1.54)	0.49 (0.17, 1.44)[h]
HPV 16				
Seronegative[a]	2609	101	1.00	1.00
Seropositive[b]	1062	40	0.98 (0.68, 1.41)	1.27(0.83, 1.92)[i]
HPV 18				
Seronegative[a]	2803	64	1.00	1.00
Seropositive[b]	1158	12	**0.45 (0.24, 0.84)**	**0.42 (0.19, 0.93)[j]**

Footnote: HPV- Human Papillomavirus, HR- Hazard ratios
a – Seropositive for any of the 4 HPV types (HPV 6, 11, 16 and 18)
b – Seronegative for all 4 HPV types (HPV 6, 11, 16 and 18)
c- Adjusted for marital status, alcohol use, lifetime male sexual partners (time varying), and new female partners in past 6-12 months (time varying).
d- Adjusted for country, lifetime male sexual partners (time varying), and new female partners in past 6-12 months (time varying).
e - Adjusted for country, lifetime female sexual partners (time varying), lifetime male sexual partners (time varying), and new female

partners in past 6-12 months (time varying).
f- Adjusted for marital status, alcohol use, lifetime male sexual partners (time varying), and new female partners in past 6-12 months (time varying).
g - Adjusted for age, alcohol use and new female partners in past 6-12 months (time varying).
h - Adjusted for new female partners in past 6-12 months (time varying) and new male partners in past 6-12 months (time varying).
i - Adjusted for age, country and new female partners in past 6-12 months (time varying)
j - Adjusted for marital status, lifetime female sexual partners (time varying), and new female partners in past 6-12 months (time varying).

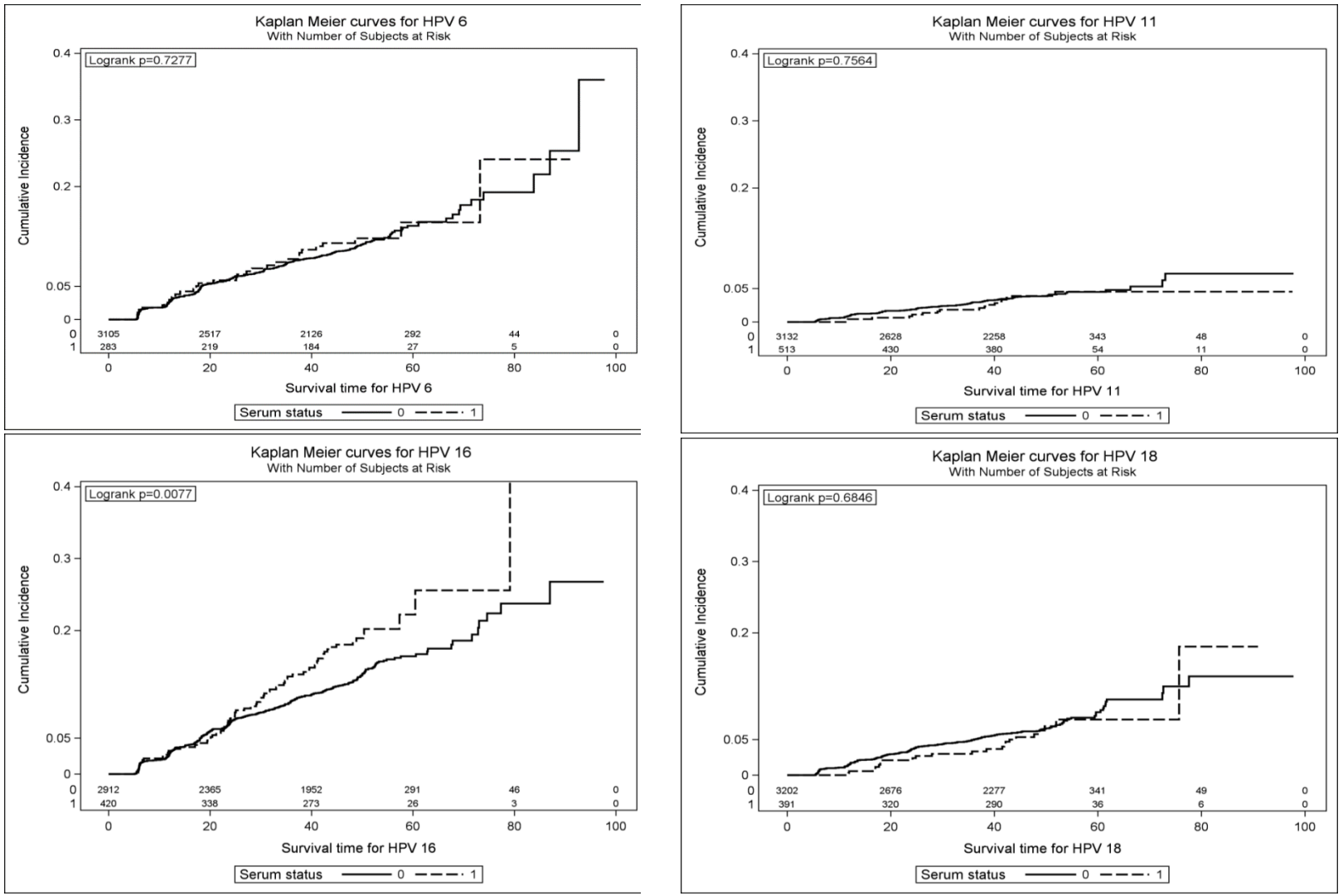

Figure 2.1 - Kaplan-Meier curves for acquisition of incident HPV 6, 11, 16, and 18 infections by serostatus among men in the HIM Study

Footnotes:

Serum status: Solid line = Seronegative subjects, Dashed line= Seropositive subjects

P values were determined using the log-rank test and denote differences across the entire follow-up period, by serum status. Values < .05 are considered statistically significant

Median failure times were 47.6 months (HPV 6), 48.6 months (HPV 11), 47.9 months (HPV 16) and 48.5 months (HPV 18).

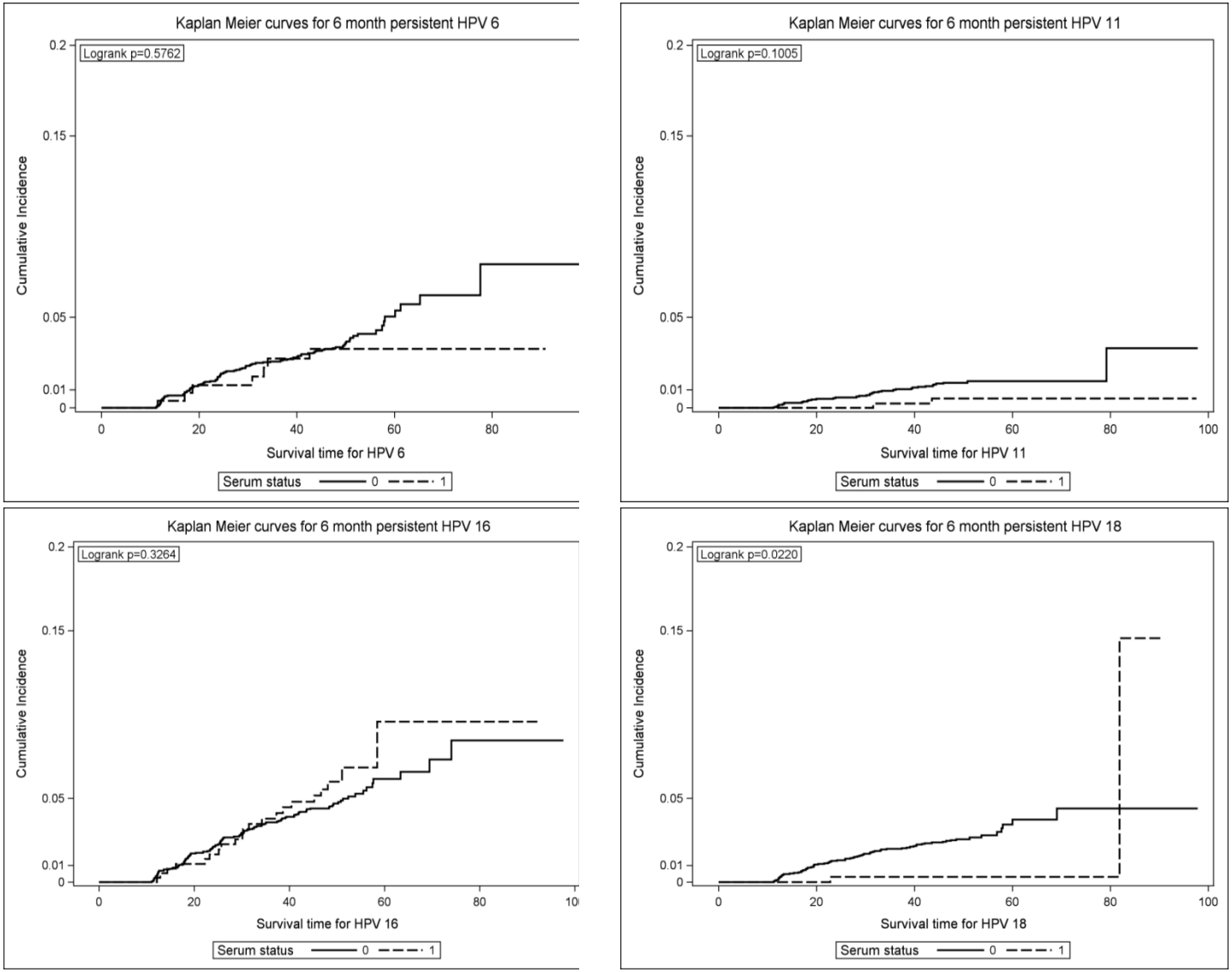

Figure 2.2 - Kaplan-Meier curves for acquisition of six-month persistent HPV 6, 11, 16, and 18 infections by serostatus among men in the HIM Study

Footnotes:

Serum status: Solid line = Seronegative subjects, Dashed line= Seropositive subjects

P values were determined using the log-rank test and denote differences across the entire follow-up period, by serum status. Values < .05 are considered statistically significant

Median failure times were 47.8 months (HPV 6), 48.7 months (HPV 11), 48.0 months (HPV 16) and 48. 6 months (HPV 18).

MANUSCRIPT TWO

Introduction:

Human Papillomavirus (HPV) infection is one of the most common sexually transmitted infections in men and women (147). The Human Papillomavirus Infection in Men (HIM) Study was among the first to report the natural history of genital HPV infection among men (147, 148). In this study, average duration of a genital HPV infection was ~7.5 months for any HPV infection and 12.2 months for HPV 16 infection (147). After initial clearance (≥ 12 month time without detection of HPV DNA), individuals were susceptible for recurrence/redetection of HPV infection with the same HPV type. Recurrent HPV infection is an important component of the natural history of these infections and may help to elucidate the anogenital HPV prevalence observed among men in numerous studies (147, 139-152).

No studies have evaluated HPV recurrence in men and only a few have examined the recurrence of cervical HPV infection among women. In studies among women, 18.1-19.5% of HPV infections were redetected (153-157). High risk sexual behaviors, such as having multiple sexual partners and new sexual partners, were reported as some of the most important risk factors associated with redetection of cervical HPV infection (153, 157). However, sexual behavior did not explain all the infection recurrences observed in women.

In the current study, we assessed recurrence of nine HPV vaccine types (HPV 6, 11, 16, 18, 31, 33, 45 52 and 58) in the HIM Study cohort (n= 4,123). Our objective was to estimate

type-specific recurrence of genital HPV infections and investigate factors associated with recurrence among men.

Methods:

Study population

The HPV Infection in Men (HIM) Study is a study of the natural history of HPV among men in Tampa, Florida (U.S.), São Paulo (Brazil), and Cuernavaca (Mexico). Detailed methods and recruitment of study participants has been described previously (148). Briefly, healthy men from each study site were followed every six months for a median follow-up time of four years. Eligibility criteria for the participants were : i) 18–70 years of age; ii) residents of one of the study sites; iii) no previous diagnosis of penile or anal cancers; iv) no previous diagnosis of genital or anal warts; v) no symptoms of a sexually transmitted infection (STI) and not receiving treatment for an STI; vi) not participating in an HPV vaccine study; vii) no history of HIV or AIDS; viii) no history of imprisonment, homelessness, or drug treatment during the past six months; and ix) willing to comply with 10 scheduled visits every six months for four years with no plans to relocate during that time. Questionnaires were administered using computer-assisted self-interviewing (CASI) at baseline and each follow-up visit to obtain extensive sexual history and health information. All eligible participants provided signed informed consent, and approval was obtained from the human subjects committees of the University of South Florida (Tampa, FL), Ludwig Institute for Cancer Research (São Paulo, Brazil), Centro de Referencia e Treinamento em Doencas Sexualmente Transmissíveis e AIDS (São Paulo, Brazil), and Instituto Nacional de Salud Publica de Mexico (Cuernavaca, Mexico).

External genital HPV DNA samples

Genital cell samples from the coronal sulcus/glans penis, penile shaft, and scrotum were collected with three prewetted Dacron swabs, which were later combined to form a single sample (147, 148). Specimens were stored at -80°C, until analyses. The QIAamp DNA Blood Mini Kit (QIAGEN) was used to extract DNA and HPV DNA tests were performed using PCR amplification of the L1 gene fragment of HPV. Thirty seven different types of genital HPV were detected with use of the Roche Linear Array assay for HPV genotyping (158). Human β-globin was tested to assess specimen adequacy, with an overall β-globin positivity of 98%.

Statistical analysis

The nine HPV types (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58) in the recently approved nonavalent HPV vaccine were included in this analysis (159). Participants with prevalent HPV infections at baseline or an incident infection during the follow-up period were eligible for inclusion in the study. Participants who were HPV DNA negative throughout the entire study period, those with prevalent infections that persisted throughout the entire study period or incident infections that persisted throughout the remaining follow-up time, and participants who dropped out of the study before infection clearance were excluded from the analysis (Figure 3.1). Two different analyses were carried out: 1) individual HPV type analysis for each of the nine genotypes (infection-level type-specific analysis), and 2) grouped analyses (recurrent infection vs. non-recurrent infection with any of the 9 vaccine types). Recurrences assessed in both of these analyses were genotype concordant (type-specific). For example, after clearance of HPV 16 infection, recurrence of HPV 16 was assessed in the analyses.

In individual HPV type (type-specific) analyses, infections were categorized into two groups: a) infections that were first observed as incident and b) infections observed as prevalent at the baseline visit. Both these groups were further categorized by infection duration; transient (observed at one visit only) and persistent (infection with duration of $\geq$ 6 months). Infection recurrence was defined as reappearance/redetection of DNA of the same HPV genotype after two successive DNA-negative tests at clinical visits scheduled at 6-month intervals ($\geq$12 months duration). Figure 1 describes the study participants excluded and included in this analysis and definitions of the analytical groups.

For each HPV genotype, the proportion of infections that recurred among individuals with a prior incident or prior prevalent infection was calculated. Visit-specific questionnaire data corresponding to the two successive 6 month study visits following the last visit when that specific HPV DNA was detected (12-month at risk period for recurrence) were utilized in analyses for men whose infections did not recur. For men whose infections did recur, questionnaire data from the visit where the recurrence was observed (as behavior was inquired for the prior 6 months) and data reported on the visit prior to the recurrence (total time period up to 12 months prior to the recurrence) were utilized in analyses. Variables of interest for these analyses included sexual behavior, tobacco, alcohol use, and factors previously shown to be associated with genital incidence and prevalence in the HIM Study cohort (147, 148).

Grouped analysis was carried out to increase the power to assess associations between overall recurrence and related risk factors. If individuals had recurrent infection of any of the 9 HPV types; they were grouped as "Recurrent infections for any type". Individuals in the "Non-recurrent infections" group did not have recurrences of any of the 9 HPV types. Subjects were further categorized into three groups: i) only incident infection with any of the nine types ii) only

prevalent infection with any of the nine types, and iii) a combination of prevalent and incident infection with any of the nine types. Demographic characteristics for these groups were compared using Chi-square tests with Monte Carlo estimation of exact p-values. In the grouped analysis men who had multiple HPV infections, "at risk period for recurrence" differed across infections in men. Therefore, we characterized individual behavior based on participants' overall pattern of responses throughout the study follow-up for sexual behavior, tobacco, and alcohol use questions. Logistic regression was utilized to evaluate the association between these variables and infection recurrence. Behavioral factors, along with demographic variables, were evaluated in adjusted models. Selection of variables for inclusion in final model was based on the Akaike information criteria (AIC) values.

Results:

Genital DNA samples were available for 4,123 participants of the HIM study at baseline. For type-specific analyses, subjects were excluded (HPV6 = 621, HPV11 =171, HPV16 = 762, HPV18 = 300, HPV31 = 226, HPV33 = 76, HPV45 = 268, HPV52 = 486, and HPV58 = 292) if they were HPV DNA negative throughout the study follow-up, had persistent infection throughout the duration of their follow-up time or dropped out before clearing the infection. After these exclusions, 1,119 men with incident and 1,106 men with prevalent infections of one or more of the nine vaccine type HPVs were included in analyses. Median duration of follow-up was 3.7 years (range 0.4-7.4 years, mean 3.6 years, IQR 3.4-4.0).

A) Individual HPV type recurrence

Overall, men whose initial infections were prevalent had higher recurrences compared to men with initial incident infections (Figure 3.2). Recurrence proportions and factors associated with recurrence of prevalent and incident infections are as follows.

Prevalent infections

Men with prior transient prevalent infections had recurrences ranging from 3.9%-31.2%, while men with prior persistent prevalent infections had recurrence ranging from 10.8%-36.8%. Among men with a prior transient prevalent infection, HPV 52 infection had the highest rate of recurrence (31.2%), followed by HPV 45 (22.9%), HPV 58 (21.4%), HPV 6 (19.8%), and HPV 16 (19.6%) [Table 3.1a]. Among men with a prior persistent prevalent infection, HPV 45 had the highest rate of recurrence (36.8%), followed by HPV 52 (32.4%), HPV 16 (23.5%), and HPV6 (21.9%). Overall, persistent prevalent infections had higher recurrences compared to prevalent infections that were initially transient. The average cleared time between the initial prevalent infection (regardless of whether it was transient or persistent) and the recurrent infection was 20.2 months for HPV 6, 17.2 months for HPV 11, 21.5 months for HPV 16, 18.7 months for HPV 18, 26.2 months for HPV 31, 22.5 months for HPV 33, 22.1 months for HPV 45, 25.4 months for HPV 52, and 21.3 months for HPV 58.

Incident infections

Recurrence of infections that were detected first as incident infections ranged from 0.0%-19.7% for transient incident infections compared to 0.0%-26.3% among men whose prior incident infections were persistent. Among men whose incident infections were initially transient, the highest rate of recurrence was for HPV 58 (19.7%), followed by HPV 52 (14.6%),

HPV 18 (12.6%) and HPV 16 (11.8%) [Table 3.1b]. Among men whose prior incident infections were persistent, the highest recurrence was observed for HPV 11 (26.3%), followed by HPV 52 (24.5%), HPV18 (17.6%) and HPV 45 (13.6%). Similar to what was observed among prevalent genital HPV infections, incident infections that were persistent had higher rates of recurrence compared to incident infections that were transient. The average time between clearance of the incident infection (regardless of whether it was transient or persistent) and detection of the recurrence was 16.8 months (HPV 6), 18.2 months (HPV 11), 18.6 months (HPV 16), 17.4 months (HPV 18), 18.7 months (HPV 31), 19.1 months (HPV 33), 21.3 months (HPV 45), 18.9 months (HPV 52), and 15.2 months (HPV 58).

Factors associated with genital HPV infection recurrence

Sexual behavior variables were consistently associated with recurrence of infection, regardless of whether the initially detected infection was prevalent or incident. Among men with initial prevalent infections, HPV 6 infection recurrence was significantly associated with having >=2 new female partners in previous 6-12 months (Table 3.2a). HPV 16 recurrence was associated with higher frequency of sexual intercourse with female partners (>30 times) in the past 6-12 months. For HPV 31, men who reported having a new female sexual partner had higher rates of recurrence (38.5%), compared to men who did not report a new sexual partner (Table 3.2b). Recurrence of HPV 58 was highest among men consuming <0.5 drinks of alcohol per day.

Among men whose initially detected infections were incident, having one or more new male sexual partners in the previous 6-12 months was significantly associated with higher recurrence of HPV 16 infections (p= 0.01, Table 3.3a). Additionally, current smokers had the highest proportion of HPV 16 recurrence (18.6%), although this association did not reach

statistical significance (p= 0.06). Significantly higher rates of HPV 52 recurrence were observed among former smokers (27.3%) compared to current smokers (p= 0.04, Table 3.3b). HPV 58 recurrence was associated with higher frequency of sexual intercourse (>30 times) with female partners in past 6-12 months, but this association did not reach statistical significance (p = 0.09).

B) HPV infection recurrence in grouped analysis

In grouped analyses, numbers of men included in each of the three groups were, 635 (only incident infection of any of the nine types), 641 (only prevalent infection of any of the nine types), and 261 (mixed infections or combination of prevalent and incident infections) [Figure 3.3]. In this analysis, 13.4% men with prior incident infections had recurrence of any of the nine HPV types. In comparison, 24.6% of men with prior prevalent infections had recurrence of any of the nine HPV types. The highest rates of recurrence were observed among men who in the past had a combination of both an incident and prevalent infections of different types (43%). White men had the highest proportion (17.6%) of incident infections that recurred (p= 0.036, Table 3.4). Recurrence of incident infections was also higher among men with a greater number of lifetime male sexual partners (p= 0.012) and a greater number of new male partners (p= 0.011). Among men with combination of incident/prevalent infections, men with circumcision had higher recurrence compared to the uncircumcised men (p= 0.011). Among men with initially prevalent infections, none of the considered variables were significantly associated with infection recurrence.

In logistic regression models (Table 3.5) utilizing overall behavior of the participants during the follow-up period, statistically significant associations (adjusted odds ratios= aOR) were observed with lifetime number of male sexual partners (aOR = 2.40, 95% CI 1.19-4.84) and

number of new male sexual partners (aOR 2.35, 95% CI 1.16-4.74) among men who previously only had an incident infection detected. Among men with a prior prevalent infection, moderate alcohol consumption (0.5-<1.5 drinks per day) was significantly associated with a higher risk of recurrence (aOR= 1.59, 95% CI 1.01, 2.53) compared to mild alcohol consumption (<0.5 drinks/day). Among men with combination of prior incident and prevalent infections, none of the considered variables yielded statistically significant results.

Discussion:

This is the first study to report recurrence of nine vaccine type (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58) genital HPV infections among men. Our results indicate that as many as 31.2% of genital HPV infections recur with the same type, with higher rates of recurrence for oncogenic HPV types (HPV 16, 18, 45, 52, and 58).

In the current analysis, a higher number of new male sexual partners was consistently associated with higher rates of recurrence of incident infections in both type-specific (HPV 16) analyses as well as grouped analyses. Other high-risk sexual behaviors (higher number of new female partners and frequency of sexual intercourse) were also associated with higher rates of recurrence of initially prevalent HPV 6, 16 and 31 infections. Recurrence significantly differed by smoking and alcohol use only for HPV types 16, 52, and 58. As no prior study has described genital HPV recurrence among men, it is unclear with the rates reported here are unique to the cohort, or geographic regions of study.

Among women, only a few studies have examined the recurrence of cervical HPV infection. One study found that 19.4% of incident infections of multiple genotypes were redetected within a year (154). A study examining specific HPV types reported a range of 0-16%

recurrent HPV infections, with up to 11% of HPV 16 infections reappearing after 3 years (155). A study by Moscicki et al. observed that HPV 16 DNA was redetected in 18.1% women after clearance of initial infection (156). This study also reported that having one or more sexual partners (aOR= 2.90, 95% CI 1.22, 6.90) and having a new sexual partner in the past eight months (aOR= 1.1, 95% CI 1.02, 1.19) were associated with increased risk of redetection (156). Another study observed an association between recurrence and having new sexual partners with relative risk (RR) estimate of 3.7 (95% CI=1.1–13.8) for all HPV types included in the analysis (153). These results are comparable to our findings of recurrence of HPV infection among men. In contrast, lower rates of recurrence were observed (7.7% women with intervening negative results and 2.7% women with definite clearance) in a study that included multiple carcinogenic HPV types (157).

We observed higher rates of recurrence among participants with prior prevalent infections and prior persistent infections (either incident or prevalent). Baseline prevalent infections had a relatively longer observation period to allow for clearance and re-detection compared infections detected as incident at some point in the follow-up period. As a result, there were more recurrence events and greater power to assess associations with infections initially detected as prevalent. This may partially explain the higher rates of recurrence for prior prevalent infections. It is also likely that individuals with pre-existing infections had high risk sexual behavior and as a result were at higher risk of re-acquiring of HPV infections. Similarly, individuals with persistent infections may have engaged in high risk sexual behaviors, but due to the small sample size, we were unable to carry out separate analyses comparing transient and persistent infections for sexual behavior variables.

There are three possible explanations for observed rates of HPV infection recurrence: a) sexual exposure from the same partner who has not cleared the infection; b) exposure to a new partner who is infected with the same HPV type; and c) reactivation of latent infections. Our results show that new male partners, new female partners, and higher numbers of lifetime sexual partners lead to higher genital HPV infection recurrence. We do not, however, have information to differentiate whether the recurrence was acquired from a new partner or from the same partner. Partner studies as well as perhaps evaluation of HPV status with next generation sequencing are needed to shed light on the origin of the recurrent infections. However, intra-typic variant analysis may not identify the source of infection, as specific HPV variants are more prevalent in a particular geographic area (160-162); thus probability of infection from a new source is highest for the prevalent variant circulating in the community. In addition, current methods can not differentiate between latent and newly acquired infections (163). Thus, analysis of recurrent infection presents a number of challenges and logistic issues.

Strengths of this study include large sample size, multinational nature of the cohort, and long follow-up time. Additionally, standard and robust HPV DNA genotyping measures were utilized (164-168). Furthermore, we carefully assessed behavior during the intervening cleared period (susceptibility period for infection re-acquisition). The study also had several limitations that may have affected our results. We did not have information regarding HPV infection status of participants prior to the study start, therefore it is likely that participants may have had acquired and cleared infections prior to entering the study. Obtaining data for prior HPV DNA status is challenging as significant cost and resources are required to obtain this information, especially among men who were sexually active for a long time. There is also a possibility of existing latent infection prior to study start or during the follow-up being detected as another

infection (163, 169-172). Latency of HPV is proposed based on the animal model whereby HPV is retained in a latent state in the basal epithelial stem cell pool (163). Current HPV DNA detection methods may not be sensitive enough to detect low viral load of latent infections (169-171) and re-activation of latent infection is less likely in immune competent subjects (as in the HIM study). Future studies are needed to further elucidate the latency of HPV infections in men and women.

Misclassification of HPV infection status is also a possibility. Low viral load and long period of time between the initial infection and assessment of HPV DNA may lead to misclassification of HPV infection status. However, such misclassification should play a very minor role in analyses, considering the robust methods used for HPV DNA detection (164-168). We did not have a sufficient number of recurrent infections to adequately power our individual type-specific analyses, especially for less common HPV types (e.g. HPV 11, 31, 33). We attempted to address this issue in our grouped analyses of all nine HPV types. Furthermore, subjects excluded from the analysis did not differ significantly from study participants for sexual behavior and other important demographic variables.

In conclusion, men are susceptible to genital HPV infection recurrence, with up to 31% of prevalent and 20% of incident infections recurring over time. Recurrence of HPV infection among men may be influenced by high-risk sexual behavior; including higher numbers of new sexual partners and lifetime sexual partners. Given the complexity of analyzing recurrence of HPV infections, future studies are needed to further understand the role of HPV recurrence in the etiology of HPV associated diseases.

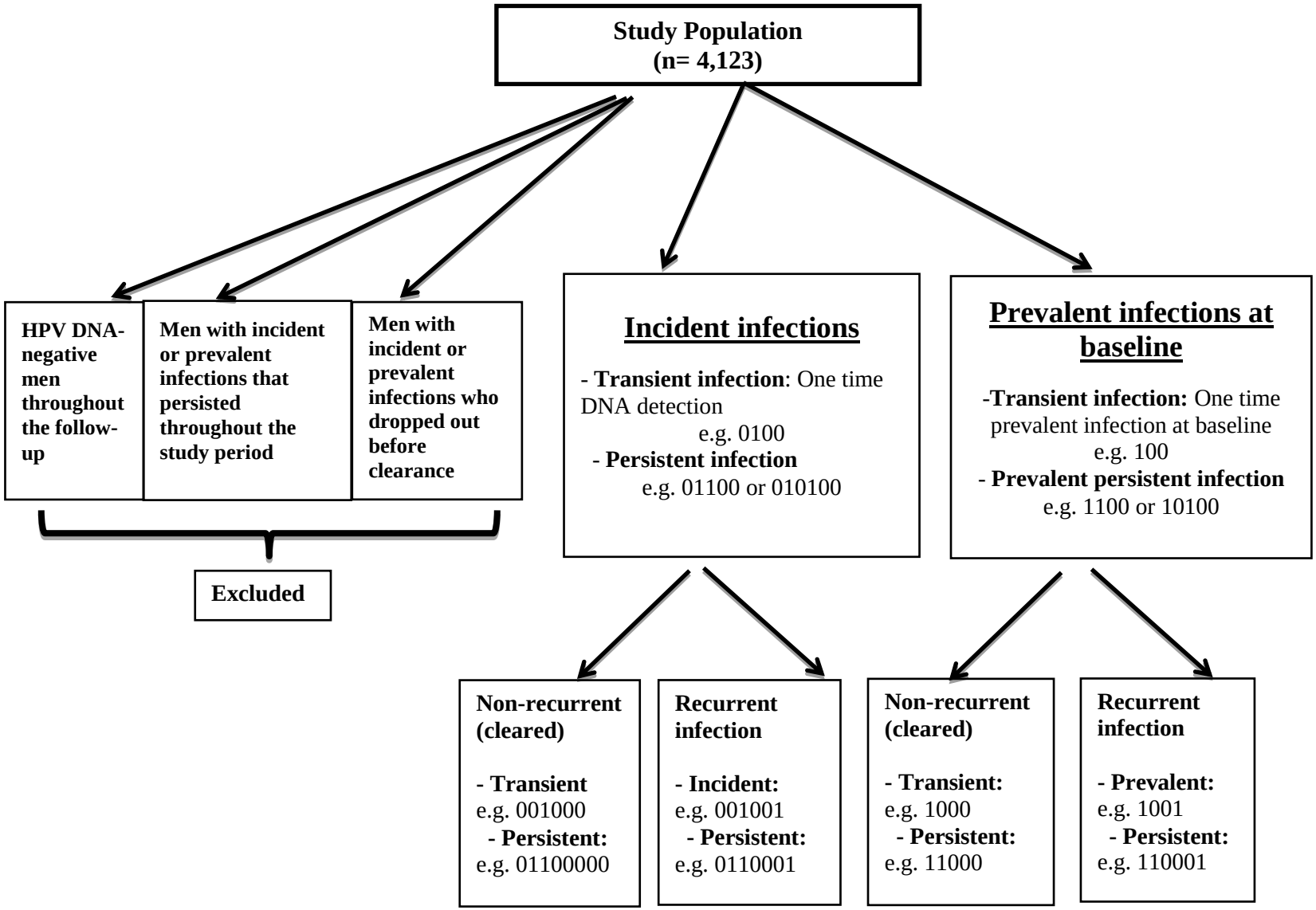

Figure 3.1: Outline for tracking recurrent infections

Footnote: In examples, 0 = HPV DNA-negative status, 1 = HPV DNA-positive status

Total numbers of excluded subjects according to HPV types were: HPV6 = 621, HPV11 =171, HPV16 = 762, HPV18 = 300, HPV31 = 226, HPV33 = 76, HPV45 = 268, HPV52 = 486, and HPV58 = 292.

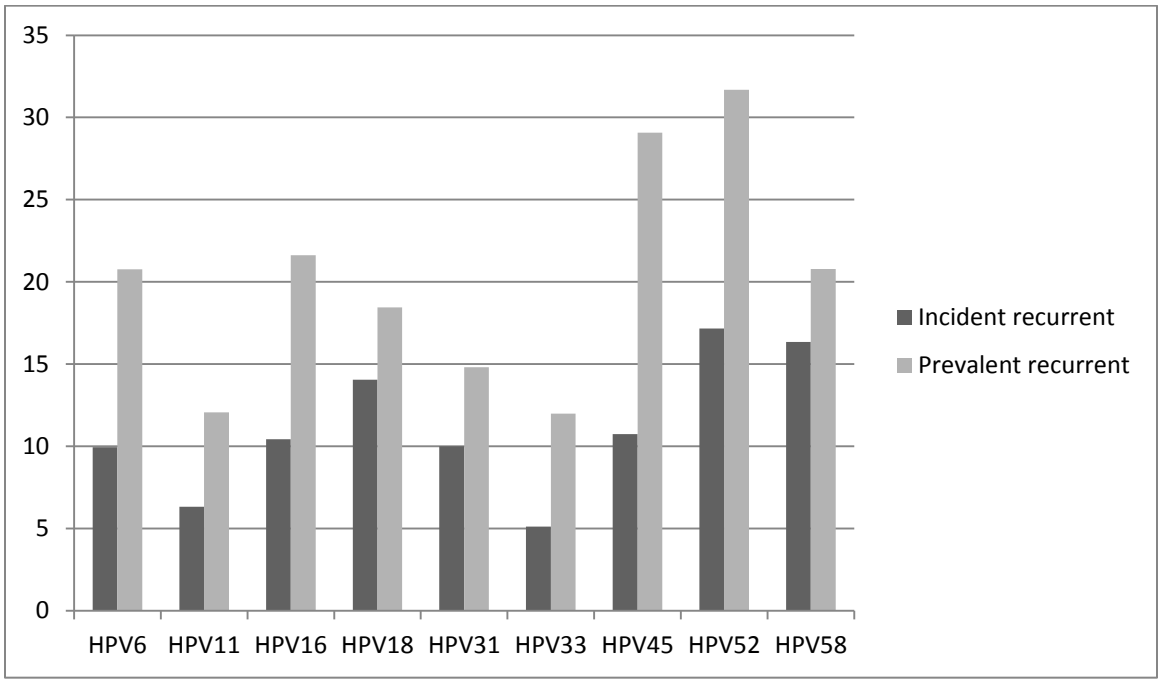

Figure 3.2: Proportion of recurrence infection among total prevalent and incident infections for HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 among men in USA, Brazil and Mexico in the HIM study.
Footnotes:

Abbreviation: HPV, human papillomavirus.

Incident Recurrent: Incident infections (either transient or persistent) that cleared (2 successive negative DNA results) and then recurred during the median follow-up time of 48 months.

Prevalent Recurrent[i] Prevalent infections (either transient or persistent) that cleared (2 successive negative DNA results) and then recurred during the median follow-up time of 48 months

Table 3.1a: Proportion of recurrent infections among men with a prior prevalent HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 infections.

HPV types	Total prevalent infections	Transient Prevalent[a] infections		Persistent Prevalent[b] infections	
		Recurrent[c] / Total transient infections	Proportion (%)	Recurrent[d] / Total persistent infections	Proportions (%)
HPV 6	260	29/146	19.86	25/114	21.93
HPV 11	58	1/26	3.85	6/32	18.75
HPV 16	282	26/133	19.55	35/149	23.50
HPV 18	103	10/57	17.54	9/46	19.57
HPV 31	81	8/44	18.18	4/37	10.81
HPV 33	25	1/13	7.69	2/12	16.67
HPV 45	86	11/48	22.92	14/38	36.84
HPV 52	161	29/93	31.18	22/68	32.35
HPV 58	101	9/42	21.43	12/59	20.34

Footnotes:

Abbreviation: HPV, human papillomavirus.

[a] **Transient prevalent infection:** A prevalent infection that was detected at only one time point

[b] **Persistent prevalent infection:** Persistence of prevalent infection for duration of 6 months or more, that cleared at some point during follow-up.

[c] **Transient prevalent recurrent**: Prevalent infections that cleared (2 successive negative DNA results) and then recurred during the follow-up period

[d] **Prevalent persistent recurrent:** Persistent prevalent infections that cleared (2 successive negative DNA results) and then recurred during the median follow-up time of 48 months.

Table 3.1b: Proportion of recurrent infections among men with a prior incident HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 infections.

HPV types	Total incident infections	Transient incident[a] infections		Incident persistent[b] infections	
		Recurrent[c] / Total incident infections	Proportions (%)	Recurrent[d] / Total persistent infections	Proportions (%)
HPV 6	211	15/164	9.15	6/47	12.77
HPV 11	79	0/60	0.00	5/19	26.32
HPV 16	249	22/195	11.28	4/54	7.41
HPV 18	121	11/87	12.64	6/34	17.65
HPV 31	90	6/66	9.09	3/24	12.50
HPV 33	39	2/33	6.06	0/6	0.00
HPV 45	121	10/99	10.10	3/22	13.64
HPV 52	204	22/151	14.57	13/53	24.53
HPV 58	104	14/71	19.72	3/33	9.09

Footnotes:

Abbreviation: HPV, human papillomavirus.

[a] **Transient incident:** An incident infection that was detected at only one time point

[b] **Incident persistent infection:** An incident infection that had an observed duration of 6 month or more.

[c] **Transient incident recurrent:** incident infections that cleared (2 successive negative results) and then recurred during the follow-up period.

[d] **Incident persistent recurrent:** Persistent incident infections that cleared (2 successive negative results) and then recurred during the median follow-up time of 48 months.

Table 3.2a - Factors associated with type specific recurrence of prior prevalent[a] HPV infections (HPV 6, 16 and 18), with sexual behaviors assessed during intervening cleared period[b]

Behavior variables	HPV 6			HPV 16			HPV 18		
	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]
Smoking									
Never	124 (79.0)	33 (21.0)	0.9734	138 (78.4)	38 (21.6)	0.6922	49 (80.3)	12 (19.7)	0.9330
Former	18 (78.3)	5 (21.7)		14 (70.0)	6 (30.0)		8 (80.0)	2 (20.0)	
Current	63 (80.8)	15 (19.2)		65 (79.3)	17 (20.7)		26 (83.9)	5 (16.1)	
Alcohol use									
0-<0.5 drinks/day	84 (84.8)	15 (15.2)	0.1820	85 (75.9)	27 (24.1)	0.7593	22 (75.9)	7 (24.1)	0.4655
0.5-<1.5 drinks/day	39 (73.6)	14 (26.4)		44 (77.2)	13 (22.8)		24 (80.0)	6 (20.0)	
≥1.5 drinks/day	80 (76.9)	24 (23.1)		85 (80.2)	21 (19.8)		36 (87.8)	5 (12.2)	
Lifetime female sexual partners									
0-1 partners	28 (80.0)	7 (20.0)	0.1523	37 (74.0)	13 (26.0)	0.7865	9 (81.8)	2 (18.2)	0.8172
2-12 partners	82 (83.7)	16 (16.3)		69 (79.3)	18 (20.7)		33 (84.6)	6 (15.4)	
>12 partners	82 (72.6)	31 (27.4)		100 (77.5)	29 (22.5)		31 (77.5)	9 (22.5)	
No. of new female sexual partners in previous 6-12 months[e]									
0 partners	91 (79.8)	23 (20.2)	**0.0007**	108 (77.1)	32 (22.9)	0.7582	45 (84.9)	8 (15.1)	0.3684
1 partners	39 (88.6)	5 (11.4)		31 (75.6)	10 (24.4)		8 (72.7)	3 (27.3)	
≥2 partners	26 (56.5)	20 (43.5)		57 (81.4)	13 (18.6)		19 (73.1)	7 (26.9)	

Table 3.2a (Continued)

Behavior variables	HPV 6			HPV 16			HPV 18		
	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]
Frequency of sexual intercourse with female partners in last 6-12 months									
0-5	43 (78.2)	12 (21.8)	0.9812	50 (70.4)	21 (29.6)	**0.0452**	21 (84.0)	4 (16.0)	0.9357
6-30	44 (78.6)	12 (21.4)		58 (87.9)	8 (12.1)		15 (78.9)	4 (21.1)	
>30	102 (77.3)	30 (22.7)		101 (76.5)	31 (23.5)		40 (81.6)	9 (18.4)	
Lifetime male sexual partners									
None	155 (77.1)	46 (22.9)	0.2740	177 (79.0)	47 (21.0)	1.0000	63 (77.8)	18 (22.2)	0.1201
≥1 partners	30 (85.7)	5 (14.3)		21 (77.8)	6 (22.2)		13 (100.0)	0 (0.0)	
No. of new male sexual partners in previous 6-12 months									
0 partners	160 (77.7)	46 (22.3)	0.8183	181 (78.7)	49 (21.3)	0.7920	68 (79.1)	18 (20.9)	0.1986
≥ 1 partners	24 (80.0)	6 (20.0)		18 (81.8)	4 (18.2)		10 (100.0)	0 (0.0)	

Footnotes:

Abbreviations: HPV, human papilloma virus, NA- Not applicable.

a – Prevalent infections included prevalent infections and prevalent infections that persisted for a duration of 6 months or more.

b – Intervening cleared period was defined as time period after clearance (two successive negative DNA detections) for the last DNA positive result for the type specific incident HPV infection. For recurrent infection it was the visit when recurrence was observed (behavior was inquired for previous 6 months) and one visit prior to recurrence (incident was cleared during this time and they were susceptible to recurrence).

c - p-values for categorical variables were calculated using the chi-square test with Monte Carlo estimation of exact p-values, $\alpha = 0.05$. p- value represents global value for overall difference between non-recurrent and recurrent infections.

Table 3.2b - Factors associated with type specific recurrence of prior prevalent[a] HPV infections (HPV 6, 16 and 18), with sexual behaviors assessed during intervening cleared period[b]

Behavior variables	HPV 31			HPV 45			HPV 52			HPV 58		
	Non-recurrent	Recurrent	p[c]	Non-recurrent	Recurrent	p[c]	Non-recurrent	Recurrent	p[c]	Non-recurrent	Recurrent	p[c]
Smoking												
Never	47 (85.5)	8 (14.5)	1.000	40 (71.4)	16 (28.6)	0.500	57 (62.0)	35 (38.0)	0.109	44 (77.2)	13 (22.8)	0.087
Former	2 (100.0)	0 (0.0)		7 (87.5)	1 (12.5)		14 (77.8)	4 (22.2)		3 (50.0)	3 (50.0)	
Current	20 (83.3)	4 (16.7)		14 (63.6)	8 (36.4)		38 (77.6)	11 (22.4)		33 (86.8)	5 (13.2)	
Alcohol use												
0-<0.5 drinks/day	28 (90.3)	3 (9.7)	0.283	28 (80.0)	7 (20.0)	0.313	45 (71.4)	18 (28.6)	0.725	28 (65.1)	15 (34.9)	**0.010**
0.5-<1.5 drinks/day	7 (70.0)	3 (30.0)		10 (62.5)	6 (37.5)		19 (67.9)	9 (32.1)		19 (90.5)	2 (9.5)	
≥1.5 drinks/day	31 (83.8)	6 (16.2)		21 (65.6)	11 (34.4)		42 (64.6)	23 (35.4)		33 (89.2)	4 (10.8)	
Lifetime female sexual partners												
0-1 partners	5 (100.0)	0 (0.0)	0.167	6 (75.0)	2 (25.0)	1.000	14 (82.4)	3 (17.6)	0.206	11 (84.6)	2 (15.4)	1.000
2-12 partners	22 (75.9)	7 (24.1)		20 (76.9)	6 (23.1)		37 (72.5)	14 (27.5)		26 (81.3)	6 (18.8)	
>12 partners	38 (88.4)	5 (11.6)		31 (75.6)	10 (24.4)		52 (62.7)	31 (37.3)		40 (80.0)	10 (20.0)	
No. of new female sexual partners in previous 6-12 months[e]												
0 partners	30 (88.2)	4 (11.8)	**0.032**	30 (75.0)	10 (25.0)	0.458	53 (72.6)	20 (27.4)	0.108	45 (83.3)	9 (16.7)	0.149
1 partners	8 (61.5)	5 (38.5)		9 (81.8)	2 (18.2)		12 (50.0)	12 (50.0)		15 (78.9)	4 (21.1)	
≥2 partners	23 (92.0)	2 (8.0)		15 (62.5)	9 (37.5)		22 (61.1)	14 (38.9)		13 (61.9)	8 (38.1)	

Table3.2b (Continued)

Behavior variables	HPV 31			HPV 45			HPV 52			HPV 58		
	Non-recurrent	Recurrent	p[c]	Non-recurrent	Recurrent	p[c]	Non-recurrent	Recurrent	p[c]	Non-recurrent	Recurrent	p[c]
Frequency of sexual intercourse with female partners in last 6-12 months												
0-5	9 (75.0)	3 (25.0)	0.649	14 (82.4)	3 (17.6)	0.577	25 (71.4)	10 (28.6)	0.103	18 (69.2)	8 (30.8)	0.204
6-30	11 (84.6)	2 (15.4)		14 (82.4)	3 (17.6)		28 (82.4)	6 (17.6)		18 (78.3)	5 (21.7)	
>30	45 (86.5)	7 (13.5)		28 (70.0)	12 (30.0)		53 (63.1)	31 (36.9)		43 (86.0)	7 (14.0)	
Lifetime male sexual partners												
None	63 (85.1)	11 (14.9)	0.403	47 (70.1)	20 (29.9)	1.000	86 (65.2)	46 (34.8)	0.182	67 (82.7)	14 (17.3)	0.108
≥1 partners	2 (66.7)	1 (33.3)		9 (7.05)	3 (25.0)		14 (82.4)	3 (17.6)		6 (60.0)	4 (40.0)	
No. of new male sexual partners in previous 6-12 months[e]												
None	63 (85.1)	11 (14.9)	1.000	49 (69.0)	22 (31.0)	0.429	88 (64.7)	48 (35.3)	0.227	68 (82.9)	14 (17.1)	0.101
≥1 partners	3 (75.0)	1 (25.0)		7 (87.5)	1 (12.5)		11 (84.6)	2 (15.4)		6 (60.0)	4 (40.0)	

Footnotes:

Abbreviations: HPV, human papilloma virus.

a – Prevalent infections included prevalent infections and prevalent infections that persisted for a duration of 6 months or more.

b – Intervening cleared period was defined as time period after clearance (two successive negative DNA detections) for the last DNA positive result for the type specific incident HPV infection. For recurrent infection it was the visit when recurrence was observed (behavior was inquired for previous 6 months) and one visit prior to recurrence (incident was cleared during this time and they were susceptible to recurrence).

c - p-values for categorical variables were calculated using the chi-square test with Monte Carlo estimation of exact p-values, $\alpha = 0.05$. p- value represents global value for overall difference between non-recurrent and recurrent infections.

Table 3.3a- Factors associated with type specific recurrence of prior incident[a] HPV infections (HPV 6, 16 and 18), with sexual behaviors assessed during intervening cleared period[b]

Behavior variables	HPV 6			HPV 16			HPV 18		
	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]
Smoking									
Never	133 (90.5)	14 (9.5)	0.7573	160 (92.0)	14 (8.0)	0.0636	69 (86.3)	11 (13.8)	0.8385
Former	4 (80.0)	1 (20.0)		11 (91.7)	1 (8.3)		2 (100.0)	0 (0.0)	
Current	51 (91.1)	5 (8.9)		48 (81.4)	11 (18.6)		31 (83.8)	6 (16.2)	
Alcohol use									
0-<0.5 drinks/day	84 (94.4)	5 (5.6)	0.1195	90 (90.0)	10 (10.0)	1.0000	44 (86.3)	7 (13.7)	1.0000
0.5-<1.5 drinks/day	35 (83.3)	7 (16.7)		48 (88.9)	6 (11.1)		18 (85.7)	3 (14.3)	
≥1.5 drinks/day	68 (88.3)	9 (11.7)		81 (89)	10 (11)		41 (85.4)	7 (14.6)	
Lifetime female sexual partners									
0-1 partners	25 (92.6)	2 (7.4)	0.8472	30 (88.2)	4 (11.8)	0.3456	5 (100.0)	0 (0.0)	0.6230
2-12 partners	68 (90.7)	7 (9.3)		74 (86.0)	12 (14.0)		27 (84.4)	5 (15.6)	
>12 partners	82 (89.1)	10 (10.9)		100 (92.6)	8 (7.4)		61 (87.1)	9 (12.9)	
No. of new female sexual partners in previous 6-12 months[e]									
0 partners	104 (92.0)	9 (8.0)	0.5831	117 (88.0)	16 (12.0)	0.4007	39 (84.8)	7 (15.2)	0.7480
1 partners	34 (87.2)	5 (12.8)		38 (86.4)	6 (13.6)		14 (93.3)	1 (6.7)	
≥2 partners	40 (87.0)	6 (13.0)		60 (93.8)	4 (6.3)		47 (87.0)	7 (13.0)	

Table 3.3a (Continued)

Behavior variables	HPV 6			HPV 16			HPV 18		
	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]
Frequency of sexual intercourse with female partners in last 6-12 months									
0-5	40 (93.0)	3 (7.0)	0.7489	51 (89.5)	6 (10.5)	1.0000	15 (83.3)	3 (16.7)	0.1587
6-30	45 (88.2)	6 (11.8)		43 (89.6)	5 (10.4)		16 (76.2)	5 (23.8)	
>30	91 (89.2)	11 (10.8)		114 (89.1)	14 (10.9)		65 (91.5)	6 (8.5)	
Lifetime male sexual partners									
None	155 (90.6)	16 (9.4)	0.2851	179 (90.9)	18 (9.1)	1.0000	86 (87.8)	12 (12.2)	0.2276
≥1 partners	20 (83.3)	4 (16.7)		20 (90.9)	2 (9.1)		11 (73.3)	4 (26.7)	
No. of new male sexual partners in previous 6-12 months									
0 partners	158 (90.8)	16 (9.2)	0.2543	187 (91.2)	18 (8.8)	**0.0111**	88 (86.3)	14 (13.7)	0.6939
≥ 1 partners	18 (81.8)	4 (18.2)		15 (71.4)	6 (28.6)		11 (78.6)	3 (21.4)	

Footnotes:

Abbreviations: HPV, human papilloma virus.

a – Incident infections include incident infections and incident infections that persisted for a duration of 6 months or more.

b – Intervening cleared period was defined as time period after clearance (two successive negative DNA detections) for the last DNA positive result for the type specific incident HPV infection. For recurrent infection it was the visit when recurrence was observed (behavior was inquired for previous 6 months) and one visit prior to recurrence (incident was cleared during this time and they were susceptible to recurrence).

c - p-values for categorical variables were calculated using the chi-square test with Monte Carlo estimation of exact p-values, $\alpha = 0.05$. p- value represents global value for overall difference between non-recurrent and recurrent infections.

Table 3.3b- Factors associated with type specific recurrence of prior incident[a] HPV infections (HPV 31, 45, 52 and 58), with sexual behaviors assessed during intervening cleared period[b]

Behavior variables	HPV 31			HPV 45			HPV 52			HPV 58		
	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]
Smoking												
Never	48 (90.6)	5 (9.4)	1.0000	78 (88.6)	10 (11.4)	1.0000	105 (78.9)	28 (21.1)	**0.0488**	54 (83.1)	11 (16.9)	0.6941
Former	7 (87.5)	1 (12.5)		4 (100.0)	0 (0.0)		8 (72.7)	3 (27.3)		5 (100.0)	0 (0.0)	
Current	24 (88.9)	3 (11.1)		26 (89.7)	3 (10.3)		53 (93.0)	4 (7.0)		26 (81.3)	6 (18.8)	
Alcohol use												
0-<0.5 drinks/day	36 (90.0)	4 (10.0)	1.0000	55 (90.2)	6 (9.8)	0.1893	60 (84.5)	11 (15.5)	0.8899	33 (78.6)	9 (21.4)	0.5321
0.5-<1.5 drinks/day	20 (90.9)	2 (9.1)		16 (100)	0 (0)		39 (81.3)	9 (18.8)		15 (88.2)	2 (11.8)	
≥1.5 drinks/day	24 (88.9)	3 (11.1)		34 (82.9)	7 (17.1)		66 (81.5)	15 (18.5)		39 (86.7)	6 (13.3)	
Lifetime female sexual partners												
0-1 partners	14 (87.5)	2 (12.5)	0.6604	17 (94.4)	1 (5.6)	0.6607	17 (70.8)	7 (29.2)	0.1218	10 (90.9)	1 (9.1)	0.9234
2-12 partners	23 (95.8)	1 (4.2)		29 (87.9)	4 (12.1)		51 (89.5)	6 (10.5)		23 (85.2)	4 (14.8)	
>12 partners	38 (90.5)	4 (9.5)		46 (86.8)	7 (13.2)		84 (81.6)	19 (18.4)		45 (83.3)	9 (16.7)	
No. of new female sexual partners in previous 6-12 months[e]												
0 partners	43 (89.6)	5 (10.4)	0.4763	48 (85.7)	8 (14.3)	0.4800	75 (80.6)	18 (19.4)	0.9020	40 (85.1)	7 (14.9)	0.6666
1 partners	11 (100.0)	0 (0.0)		20 (95.2)	1 (4.8)		27 (84.4)	5 (15.6)		13 (76.5)	4 (23.5)	
≥2 partners	24 (85.7)	4 (14.3)		30 (90.9)	3 (9.1)		53 (82.8)	11 (17.2)		32 (86.5)	5 (13.5)	

Table 3.3b (Continued)

Behavior variables	HPV 31			HPV 45			HPV 52			HPV 58		
	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]	Non-recurrent	Recurrent	p-value[c]
Frequency of sexual intercourse with female partners in last 6-12 months												
0-5	20 (83.3)	4 (16.7)	0.5339	32 (91.4)	3 (8.6)	0.6701	32 (78.0)	9 (22.0)	0.6089	18 (94.7)	1 (5.3)	0.0886
6-30	14 (93.3)	1 (6.7)		20 (90.9)	2 (9.1)		44 (86.3)	7 (13.7)		21 (91.3)	2 (8.7)	
>30	40 (93.0)	3 (7.0)		40 (85.1)	7 (14.9)		78 (82.1)	17 (17.9)		42 (76.4)	13 (23.6)	
Lifetime male sexual partners												
None	58 (92.1)	5 (7.9)	0.3575	68 (88.3)	9 (11.7)	0.5014	124 (82.1)	27 (17.9)	0.4253	72 (83.7)	14 (16.3)	1.0000
≥1 partners	13 (81.3)	3 (18.8)		31 (93.9)	2 (6.1)		25 (89.3)	3 (10.7)		7 (77.8)	2 (22.2)	
No. of new male sexual partners in previous 6-12 months												
0 partners	59 (92.2)	5 (7.8)	0.3396	73 (89.0)	9 (11.0)	1.0000	129 (82.7)	27 (17.3)	0.2566	71 (83.5)	14 (16.5)	1.0000
≥ 1 partners	13 (81.3)	3 (18.8)		27 (90.0)	3 (10.0)		26 (92.9)	2 (7.1)		9 (81.8)	2 (18.2)	

Footnotes:

Abbreviations: HPV, human papilloma virus.

a – Incident infections include incident infections and incident infections that persisted for a duration of 6 months or more.

b – Intervening cleared period was defined as time period after clearance (two successive negative DNA detections) for the last DNA positive result for the type specific incident HPV infection. For recurrent infection it was the visit when recurrence was observed (behavior was inquired for previous 6 months) and one visit prior to recurrence (incident was cleared during this time and they were susceptible to recurrence).

c - p-values for categorical variables were calculated using the chi-square test with Monte Carlo estimation of exact p-values, $\alpha = 0.05$. p- value represents global value for overall difference between non-recurrent and recurrent infection

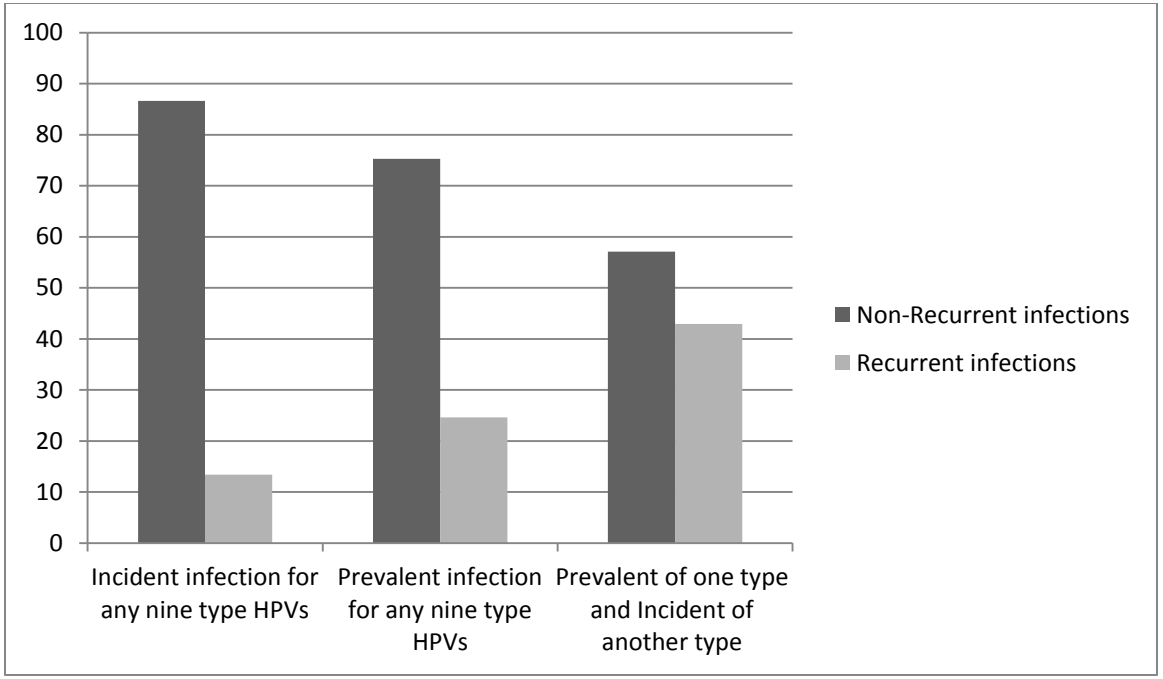

Figure 3.3: Proportions of incident and prevalent infection for nine vaccine type HPVs (6, 11, 16, 18, 31, 33, 45, 52, and 58) according to recurrence status in grouped analyses.

Footnotes:

Abbreviation: HPV, human papillomavirus

Table 3.4- Demographic characteristics comparing incident infections that recur or do not recur and prevalent infections that recur or do not recur for any of the 9 type of HPVs (HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58) among men in the HIM study.

Demographic variables	Incident infections			Prevalent infections			Prevalent infection for one type and incident for another type		
	Non-Recurrent N (%)	Recurrent N (%)	p-value[a]	Non-Recurrent N (%)	Recurrent N (%)	p-value[a]	Non-Recurrent N (%)	Recurrent N (%)	p-value[a]
Country									
United States	144 (84.2)	27 (15.8)	0.1383	139 (74.7)	47 (25.3)	0.7281	30 (51.7)	28 (48.3)	0.3084
Brazil	246 (85.4)	42 (14.6)		190 (74.2)	66 (25.8)		83 (56.1)	65 (43.9)	
Mexico	160 (90.9)	16 (9.1)		154 (77.4)	45 (22.6)		36 (65.5)	19 (34.5)	
Age									
18-30	255 (87.0)	38 (13.0)	0.7751	243 (78.9)	65 (21.1)	0.0892	90 (58.8)	63 (41.2)	0.8225
31-44	215 (87.0)	32 (13.0)		188 (73.2)	69 (26.8)		46 (54.8)	38 (45.2)	
45-73	80 (84.2)	15 (15.8)		52 (68.4)	24 (31.6)		13 (54.2)	11 (45.8)	
Marital status									
Single	240 (84.8)	43 (15.2)	0.4867	222 (77.6)	64 (22.4)	0.4352	84 (57.5)	62 (42.5)	0.7864
Married/Cohabiting	307 (88.0)	42 (12.0)		209 (73.1)	77 (26.9)		50 (58.1)	36 (41.9)	
Divorced/Separated/Widowed	50 (89.3)	6 (10.7)		49 (75.4)	16 (24.6)		14 (50.0)	14 (50.0)	
Race									
Whites	234 (82.4)	50 (17.6)	**0.0359**	227 (73.2)	83 (26.8)	0.4273	72 (56.7)	55 (43.3)	0.9721
African-Americans	112 (88.9)	14 (11.1)		70 (77.8)	20 (22.2)		35 (56.5)	27 (43.5)	
Asians/Pacific islander	11 (91.7)	1 (8.3)		7 (63.6)	4 (36.4)		2 (66.7)	1 (33.3)	
Mixed race/Others[b]	186 (91.2)	18 (8.8)		172 (78.2)	48 (21.8)		38 (59.4)	26 (40.6)	

Table 3.4 (Continued)

Demographic variables	Incident infections			Prevalent infections			Prevalent infection for one type and incident for another type		
	Non-Recurrent N (%)	Recurrent N (%)	p-value[a]	Non-Recurrent N (%)	Recurrent N (%)	p-value[a]	Non-Recurrent N (%)	Recurrent N (%)	p-value[a]
Education									
≤ 12 years	273 (86.4)	43 (13.6)	0.7707	230 (73.7)	82 (26.3)	0.1634	78 (58.6)	55 (41.4)	0.8409
13-15 years	141 (85.5)	24 (14.5)		112 (81.8)	25 (18.2)		30 (54.5)	25 (45.5)	
≥16 years	135 (88.2)	18 (11.8)		140 (74.1)	49 (25.9)		40 (55.6)	32 (44.4)	
Alcohol use*									
Non-drinkers	174 (86.6)	27 (13.4)	0.9887	204 (79.7)	52 (20.3)	0.1014	38 (64.4)	21 (35.6)	0.2436
<0.5 drinks/day	157 (86.3)	25 (13.7)		114 (71.3)	46 (28.8)		38 (50.0)	38 (50.0)	
0.5-2 drinks/day	219 (86.9)	33 (13.1)		165 (73.3)	60 (26.7)		73 (57.9)	53 (42.1)	
Cigarette smoking*									
Never	352 (85.4)	60 (14.6)	0.3288	319 (76.3)	99 (23.7)	0.6832	82 (54.3)	69 (45.7)	0.5879
Former smoker	30 (93.8)	2 (6.3)		34 (75.6)	11 (24.4)		8 (57.1)	6 (42.9)	
Current smoker	168 (88.0)	23 (12.0)		130 (73.0)	48 (27.0)		59 (61.5)	37 (38.5)	
Circumcision									
No	357 (87.7)	50 (12.3)	0.3357	320 (75.1)	106 (24.9)	0.9233	109 (62.6)	65 (37.4)	**0.0111**
Yes	193 (84.6)	35 (15.4)		163 (75.8)	52 (24.2)		40 (46.0)	47 (54.0)	
Sexual orientation									
MSW	380 (86.4)	60 (13.6)	0.8233	340 (74.7)	115 (25.3)	0.7534	105 (57.7)	77 (42.3)	0.3327
MSM	29 (82.9)	6 (17.1)		20 (76.9)	6 (23.1)		9 (75.0)	3 (25.0)	
MSWM	70 (85.4)	12 (14.6)		69 (78.4)	19 (21.6)		21 (51.2)	20 (48.8)	

Table 3.4 (Continued)

Demographic variables	Incident infections			Prevalent infections			Prevalent infection for one type and incident for another type		
	Non-Recurrent N (%)	Recurrent N (%)	p-value[a]	Non-Recurrent N (%)	Recurrent N (%)	p-value[a]	Non-Recurrent N (%)	Recurrent N (%)	p-value[a]
Lifetime female sexual partners*									
0-1 partners	66 (82.5)	14 (17.5)	0.4199	68 (81.0)	16 (19.0)	0.3773	13 (65.0)	7 (35.0)	0.1971
2-12 partners	221 (88)	30 (12)		187 (76.0)	59 (24.0)		48 (64.0)	27 (36.0)	
>12 partners	253 (87.2)	37 (12.8)		215 (73.6)	77 (26.4)		82 (52.6)	74 (47.4)	
No. of new female sexual partners*									
0 partners	210 (84.0)	40 (16.0)	0.2127	211 (77.0)	63 (23.0)	0.4468	42 (61.8)	26 (38.2)	0.6439
1 partners	205 (88.0)	28 (12.0)		150 (73.2)	55 (26.8)		54 (56.8)	41 (43.2)	
≥2 partners	131 (89.7)	15 (10.3)		94 (71.8)	37 (28.2)		50 (54.3)	42 (45.7)	
Lifetime male sexual partners*									
None	466 (88.3)	62 (11.7)	**0.0116**	392 (74.0)	138 (26.0)	0.3708	119 (56.9)	90 (43.1)	0.9426
1-12	34 (87.2)	5 (12.8)		31 (79.5)	8 (20.5)		15 (57.7)	11 (42.3)	
>12 partners	35 (72.9)	13 (27.1)		30 (83.3)	6 (16.7)		11 (52.4)	10 (47.6)	
No. of new male partners*									
0 partners	479 (88.4)	63 (11.6)	**0.0111**	403 (74.6)	137 (25.4)	0.8566	117 (56.8)	89 (43.2)	0.8745
1 partners	22 (81.5)	5 (18.5)		30 (73.2)	11 (26.8)		28 (54.9)	23 (45.1)	
≥2 partners	36 (73.5)	13 (26.5)		22 (78.6)	6 (21.4)		0 (0.00)	0 (0.00)	

Footnotes:

Abbreviations: HPV, human papilloma virus; MSM, men who have sex with men; MSW, men who have sex with women; MSWM, men who have sex with women and men

* These variables are based on overall behavior pattern of an individual during the 4 year follow-up time. An individual with most frequent behavior (e.g. 0-1 lifetime partners) during the entire follow-up was assigned in that particular category. There will be multiple intervening time period (susceptibility period for recurrence) for multiple HPV type infections and analysis based on these different intervals is not possible here.

a - p-values for categorical variables were calculated using the chi-square test with Monte Carlo estimation of p-values, $\alpha = 0.05$. p- value represents global value for overall difference between incident and recurrent infections.

b - Includes mixed race, American Indians, Alaska natives, Native Hawaiians or other races

Table 3.5 – Odds ratio and 95% confidence intervals for recurrent infections by demographic characteristics for any of the 9 type of HPVs (HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58) among men in the HIM study

		Incident infections		Prevalent infections		Prevalent of one type and incident of another type	
Variables*	**Strata**	**Crude OR and 95% CI**	**Adjusted OR[a] and 95% CI**	**Crude OR and 95% CI**	**Adjusted OR and 95% CI**	**Crude OR and 95% CI**	**Adjusted OR and 95% CI**
Alcohol use							
	0-<0.5	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
	0.5-<1.5	1.03 (0.57 1.84)	1.01 (0.55, 1.91)[a]	**1.58 (1.00, 2.50)**	**1.59 (1.01, 2.53)**[c]	1.81 (0.90, 3.63)	1.97 (0.96, 4.06)[d]
	≥1.5	0.97 (0.56, 1.68)	0.90 (0.50, 1.60)[a]	1.43 (0.93, 2.18)	1.40 (0.92, 2.15)[c]	1.31 (0.69, 2.49)	1.22 (0.63, 2.36)[d]
Cigarette smoking							
	Never	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
	Former	0.39 (0.09, 1.68)	0.43 (0.10, 1.87)[a]	1.04 (0.51, 2.13)	1.06 (0.52, 2.17)[c]	0.88 (0.30, 2.69)	0.89 (0.29, 2.76)[d]
	Current	0.80 (0.48, 1.34)	0.73 (0.42, 1.30)[a]	1.19 (0.80, 1.78)	1.21 (0.81, 1.81)[c]	0.75 (0.44, 1.26)	0.80 (0.46, 1.39)[d]
Lifetime female sexual partners							
	0-1 partners	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
	2-12 partners	0.64 (0.32, 1.28)	0.73 (0.32, 1.66)[a]	1.34 (0.72, 2.49)	1.39 (0.74, 2.59)[c]	1.05 (0.37, 2.93)	1.13 (0.39, 3.25)[d]
	>12 partners	0.69 (0.35, 1.35)	0.85 (0.38, 1.92)[a]	1.52 (0.83, 2.78)	1.50 (0.82, 2.75)[c]	1.68 (0.64, 4.43)	1.68 (0.63, 4.44)[d]
No. of new female sexual partners							
	0 partners	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
	1 partners	0.72 (0.43, 1.21)	0.91 (0.51, 1.62)[a]	1.22 (0.80, 1.86)	1.23 (0.81, 1.87)[c]	1.23 (0.65, 2.32)	1.14 (0.60, 2.19)[d]
	≥2 partners	0.60 (0.32, 1.13)	0.69 (0.35, 1.35)[a]	1.32 (0.82, 2.12)	1.29 (0.79, 2.08)[c]	1.36 (0.72, 2.57)	1.19 (0.61, 2.33)[d]
Frequency of intercourse with female partners							
	0-5	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
	6-30	0.58 (0.31, 1.09)	0.93 (0.39, 2.23)[a]	1.22 (0.73, 2.06)	1.24 (0.74, 2.10)[c]	1.01 (0.48, 2.14)	1.03 (0.47, 2.21)[d]
	>30	0.72 (0.40, 1.31)	1.27 (0.53, 3.04)[a]	1.12 (0.67, 1.86)	1.12 (0.68, 1.87)[c]	1.06 (0.54, 2.10)	0.99 (0.49, 1.98)[d]

Table 3.5 (Continued)

		Incident infections		Prevalent infections		Prevalent of one type and incident of another type	
Variables*	**Strata**	**Crude OR and 95% CI**	**Adjusted OR[a] and 95% CI**	**Crude OR and 95% CI**	**Adjusted OR and 95% CI**	**Crude OR and 95% CI**	**Adjusted OR and 95% CI**
Lifetime male sexual partners							
	None	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
	1-12 partners	1.11 (0.42, 2.93)	1.02 (0.38, 2.73)[b]	0.73 (0.33, 1.63)	0.71 (0.32, 1.60)[c]	0.97 (0.43, 2.21)	1.12 (0.48, 2.62)[d]
	>12 partners	**2.79 (1.40, 5.56)**	**2.40 (1.19, 4.84)[b]**	0.57 (0.23, 1.39)	0.53 (0.21, 1.32)[c]	1.20 (0.49, 2.95)	1.26 (0.50, 3.20)[d]
No. of new male sexual partners							
	0 partners	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
	1 partners	1.73 (0.63, 4.73)	1.19 (0.39, 3.61)[b]	1.08 (0.53, 2.21)	1.04 (0.50, 2.15)[c]	1.08 (0.58, 2.00)	1.18 (0.62, 2.26)[d]
	≥2 partners	**2.75 (1.38, 5.46)**	**2.35 (1.16, 4.74)[b]**	0.80 (0.32, 2.02)	0.76 (0.30, 1.94)[c]	0.00 (NE)	0.00 (NE)

Footnotes: HPV- Human papillomavirus, OR-Odds ratio, NE-Not estimable

* This variables are based on overall behavior pattern of an individual during the 4 year follow-up time. An individual with most frequent behavior (e.g. 0-1 lifetime partners) during the entire follow-up was assigned in that particular category. There will be multiple intervening time period (susceptibility period for recurrence) for multiple HPV type infections. To address this issue, variables were created based on overall behavior pattern instead of based on intervening time interval.

a- Adjusted for race and lifetime male sexual partners.

b- Adjusted for race.

c- Adjusted for country.

d- Adjusted for country and circumcision status.

MANUSCRIPT THREE

Introduction:

Few studies have reported the natural history of anal HPV infections among men (173-175). Prevalence of anal HPV was observed to be 47.2 % among men who have sex with men (MSM) and 12.2% among men who have sex with women (MSW) for at least one of 37 HPV types evaluated (174). Presence of anal HPV among exclusive MSW was an interesting finding which raised questions as to how infection at this anatomic site occurs. One of the proposed mechanisms is auto-inoculation whereby genital HPV infection is transferred by the heterosexual men to the anal epithelium.

Only few studies have evaluated concurrent and sequential cervical and anal HPV infections among women (176-181). Results of these studies suggested high risk (relative risk as high as 14.2) of anal HPV infection among women with previous cervical HPV infections of high risk HPV types (177). Acquisition of anal HPV infections among women was also associated with having anal sex (177, 178, 181), which is not a likely explanation for heterosexual men. Furthermore, receiving anal sex did not entirely explain the high rate of sequential cervical to anal HPV infection among women. Thus, auto-inoculation of previous cervical infection may also lead to subsequent anal HPV infection observed among women.

To date studies examining sequential genital-anal infections have not been conducted among men. In the current study, we assessed type-specific (genotype concordant) sequential acquisition of anal HPV infection following a genital HPV infection for nine vaccine HPV types

(HPV 6, 11, 16, 18, 31, 33, 45 52 and 58) among MSW participants of the HIM study cohort. We also investigated factors associated with incident anal infections following a type-specific genital HPV infection.

Methods:

Study population

The HIM study recruited participants from Tampa, Florida (U.S.), São Paulo (Brazil), and Cuernavaca (Mexico) from 2005-2009. Study methodology and recruitment of participants has been described in detail previously (182, 183). In brief, 4,123 healthy men from three study sites were followed every six months for a median follow-up time of four years. Eligibility criteria for the participants were : a) 18–70 years of age; b) residents of one of the study sites; c) no previous diagnosis of penile or anal cancers; d) no previous diagnosis of genital warts; e) no symptoms of a sexually transmitted infection (STI) and not receiving treatment for an STI; f) not participating in an HPV vaccine study; g) no history of HIV or AIDS; h) no history of imprisonment, homelessness, or drug treatment during the past six months; and i) willingness to comply with 10 scheduled visits every six months for four years with no plans to relocate during that time. From these study participants, consent was obtained for collection of anal canal exfoliated cell samples. Computer-assisted self-interviewing (CASI) was used to administer questionnaires to obtain extensive sexual history and demographic characteristics. Signed informed consent was obtained from all eligible participants, and approval was obtained from the human subjects committees of the University of South Florida (Tampa, FL), Ludwig Institute for Cancer Research (São Paulo, Brazil), Centro de Referencia e Treinamento em Doencas Sexualmente

Transmissíveis e AIDS (São Paulo, Brazil), and Instituto Nacional de Salud Publica de Mexico (Cuernavaca, Mexico).

External genital and anal HPV DNA samples

Genital cell samples from the coronal sulcus/glans penis, penile shaft, and scrotum were collected with three prewetted Dacron swabs, which were later combined to form a single genital sample (182, 183). After collection of genital specimens, 360° of the anal epithelium was swabbed between the anal os and the anal canal dentate line using a separate swab, among participants who provided consent for anal specimen collection (173-175). These swabs were placed in specimen transport medium and stored at -80°C until analyses. Genital and anal HPV DNA was extracted from these samples by the QIAamp DNA Blood Mini Kit (QIAGEN). For both genital and anal samples, PCR and HPV genotyping were performed using the Roche Linear Array assay to detect 37 HPV genotypes (184). Human β-globin was tested to assess specimen adequacy, with an overall β-globin positivity of 98%.

Statistical analysis

All HPV types in the 9-valent vaccine (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58) were assessed for sequential type-specific genital-anal infections. From the HIM study cohort, 81% of the men who have sex with women (MSW) consented for collection of anal canal specimens at baseline and the first follow-up visit (173). Participants with both genital and anal HPV DNA samples at baseline and at least one follow-up visit were included in this analysis. Men who have sex with men (MSM), men who have sex with men and women (MSWM), and subjects with anal HPV DNA-positive results at baseline were excluded from the genital-anal sequential analyses.

Eligible subjects were assessed for incident anal HPV infection throughout the follow-up period at each 6 month clinical visit. Subjects with an incident anal HPV infection were categorized into four groups for evaluation of type-specific sequential genital to anal HPV infections (Figure 4.1). These groups were: a) Subjects with genital HPV-positive results at the anal HPV-positive visit and genital HPV-negative results in previous visits; b) Subjects with genital HPV-negative results at the anal HPV-positive visit and genital HPV-negative results in previous visits; c) Subjects with genital HPV-negative results at the anal HPV-positive visit and genital HPV-positive results in previous visits; d) Subjects with genital HPV-positive results at the anal HPV-positive visit and with genital HPV-positive results in previous visits. Subjects in group a) had concurrent genital and anal infections and were not included in type-specific sequential analyses. Subjects without an incident anal HPV infection were also categorized based on prior genital HPV infection status constituting the at risk population for incidence rate calculations. Characteristics of participants who were susceptible for an incident anal HPV infection were compared across prior genital HPV infection status using Chi-square tests and Monte Carlo estimation of exact p-values.

Anal HPV infection incidence rates and 95% confidence intervals (CI) were calculated among men with a prior genital HPV infection and among men with no prior genital HPV infection. Incidence rate ratios (IRR) were calculated for each of the nine HPV types comparing prior genital and no prior genital infection groups. Grouped analyses were also carried out for any of the nine types, low risk HPV types (HPV 6, 11) and high risk HPV types (HPV 16, 18, 31, 33, 45, 52, and 58). Separate analyses were conducted to assess incidence rates of concurrent genital and anal HPV infections (excluded from the sequential analyses). We also examined

whether a prior anal infection is associated with incidence of a subsequent genital HPV infection by calculating IRR and 95% CI.

Risk of sequential detection of anal HPV infections following type-specific genital infections was assessed using a Cox proportional hazards model by estimating crude and adjusted hazard ratios (HR). Bivariate analyses (adjusting for one co-variable at a time) were carried out in HPV individual type analyses. Demographic variables and time varying covariates (alcohol use, smoking, sexual behavior variables) were evaluated in adjusted models. Sample size was too small to allow for adjustment of multiple covariates at the same time. Grouped analyses for any of the nine types, low-risk types and high-risk HPV types were carried out to increase the statistical power to assess risk of sequential genital-anal infections. Demographic and sexual behavior variables were evaluated in both backward and forward stepwise models for grouped HPV analyses. Final models included variables using the best-fit approach based on the lowest Akaike information criterion (AIC) values.

Results:

From the original HIM study cohort of 4,123 men, anal and genital HPV DNA specimens for baseline and at least one follow-up visit were available for 2,285 men. Men with prevalent anal HPV infections (n=175) and those who reported either anal or oral sex with a man at any time through the study follow-up (n=762) were excluded from the analyses. Subjects excluded from the analyses were younger, had a lower education level, and were more likely to be MSM/MSWM compared to subjects included in the current analyses. After these exclusions, 1,348 MSW were eligible for inclusion in the current study. A total of 40 incident anal HPV infections were detected during the follow-up period, with 85% of the incident anal infections

detected during the first follow-up visit. The median follow-up time in this sub-cohort was 6.8 months (mean 8.4 months, Inter quartile range: IQR 6.5-7.4).

At baseline, 660 men had no prior genital HPV infection and 688 men had a prior genital HPV infection with any of the nine HPV types evaluated (Table 4.1). These two groups differed significantly by country, with men from Brazil constituting the highest proportion (64.6%) of men with a prior genital HPV infection. The two groups also differed by race, with African-Americans (61.9%) constituting the highest proportion of men with a prior genital infection. In addition, men with prior genital HPV infection reported higher (>2 drinks/day) alcohol use (p=0.001), greater number of lifetime female sexual partners (10-49 partners, p <0.001), and greater number of new female sexual partners in the previous 6 months (≥2 partners, p=0.001).

In individual HPV type analyses, men with HPV 16 genital infections had more than a 4-fold increased risk of acquiring a subsequent anal HPV 16 infection (Incident rate ratio- IRR= 4.49, 95% CI 1.14, 18.59) [Table 4.2]. Similarly, risk of acquisition of anal HPV infection was significantly higher for HPV 52 (IRR=15.46, 95% CI 2.53, 162.33) and HPV 58 (IRR=22.89, 95% CI 3.28, 253.09) among men with a prior same type infection at the genitals. In grouped analyses, men with prior high risk genital HPV infections had more than a 2-fold increased risk of detection of a subsequent anal HPV infection compared to men with no prior genital infections (IRR = 2.61, 95% CI 1.20-6.00, Table 4.2). Overall, men with prior genital HPV infections had higher incidence rates of subsequent anal HPV infections. Incident rates for concurrent genital and anal HPV infection were low for HPV 18 (IR= 0.18, 95% CI 0.05-0.73) and HPV 45 (IR= 0.18, 95% CI 0.4-0.72), and no concurrent infections were observed for other HPV types. In contrast, prior anal infections were not associated with risk of subsequent genital

infections (IRR 1.18; 95% CI, 0.64-2.01 for high risk type HPVs and IRR 0.72; 95% CI, 0.09-2.65 for HPV 16).

In Cox proportional hazard analyses, statistically significant HRs for anal HPV infection after a genital infection were observed for HPV 16 (HR=4.63, 95% CI 1.41-15.23), HPV 52 (HR=15.78, 95% CI 3.04-81.92) and HPV 58 (HR=19.60, 95% CI 3.56-107.79) [Table 4.3]. These HRs remained statistically significant even after adjusting for demographic and time varying covariates one at a time (Table 4.3 and Appendix Table 4.1, 4.1b). Similar to the IRR analyses, a higher but non-significant HR was observed for HPV 6 (HR=4.6, 95% CI 0.93-22.84) for detection of anal HPV infection after a genital infection. In grouped HPV analyses, significantly higher adjusted hazard ratios (aHR) were observed for infection with any of the nine HPV types (aHR= 2.8, 95% CI1.32-5.99), low risk HPV types (aHR=5.89, 95% CI1.29, 27.01), and high risk HPV types (aHR=2.65, 95% CI 1.26, 5.55). Infection with any of the nine HPV types, low risk HPV types and high risk HPV types were also evaluated by adjusting one variable at a time with HRs remaining significant after adjustment for demographic and time varying covariates (Table 4.3 and Appendix Table 4.1a, 4.1b).

Discussion:

This is the first study to report sequential type-specific genital and anal HPV infections among MSW for the HPV types included in the 9-valent vaccine (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58). Overall, having a prior genital HPV infection increased the risk of a subsequent anal infection of the same HPV type, and this association was unaffected by adjusting for sexual behavior. Men with a prior genital HPV 16 infection had more than a four-fold increased risk of anal HPV 16 infection compared to men with no prior genital HPV 16 infections. Significantly higher risk of sequential anal infection after a genital infection were observed in combined

analyses for high risk HPV types, low risk HPV types, and any of the nine HPV types targeted by vaccine.

In previous publications, anal HPV infections were detected among MSW with a prevalence of 12.2% for any HPV type and 2.2% for HPV 16 (174). It is not clear how MSW acquire these infections. Our study provides the first evidence of auto-inoculation of genital infections as a possible mechanism. Among women, only two studies have assessed sequential acquisition of type-specific anal HPV infection from a prior cervical infection. A study in the Hawaii HPV cohort found a high risk for sequential cervical to anal HPV infection with an aHR of 17.33 (95% CI 10.22–29.39) for any type and an aHR of 13.25 (95% CI 3.43–51.22) for HPV types 16 and 18 (176). Another study from the same cohort also found a high risk for sequential acquisition of anal infections after cervical infection, with a relative risk (RR) of 20.5 (95% CI: 16.3-25.7) for any HPV type and a RR of 14.2 (9.86-20.5) for high risk HPV types (177). Other studies have also indicated associations between anal and cervical HPV infections, although they did not conduct sequential analyses. A study found high concurrence rate of anal and cervical HPV infections with an adjusted odds ratio (aOR) of 3.3 (95% CI, 2.5-4.4) (178). In a study among women in American Samoa, authors' found that women with anal HPV infections were more likely to have a history of prior cervical infections (aOR = 3.32, 95% CI 1.10–10.00) (179). Another study found that anal HPV infections were associated with cervical HPV infection with a relative risk (RR) = 1.3 (95% CI, 1.1–1.4) (180). A concordance rate of 20% for anal HPV infections among women with pre-existing cervical HPV infections was observed in another study (181).

In this study, risk of sequential acquisition of anal HPV infections was unaffected by sexual behavior and other demographic characteristics. Our analyses included exclusively MSW subjects and excluded any subjects who reported either oral or anal sex with men throughout the follow-up period. As number of female sexual partners and frequency of sexual intercourse with women did not explain presence of anal HPV infections, a likely explanation of these infections is auto-inoculation through prior genital infections. Auto-inoculation from genital infections may occur through contact of the hand, toilet paper, undergarments, towel, or other objects. Digital contact through female sexual partners is also a possibility, but we did not have information available to assess such transmission. Absence of other explanatory factors and significantly high risk of sequential infections observed in our analyses indicate that auto-inoculation is a likely explanation.

To the best of our knowledge, no other study has assessed the risk of type-specific sequential genital-anal infections among heterosexual men. Strengths of the current study are large sample size, multinational nature of the cohort, and standard HPV DNA genotyping methods (190-194). Apart from these strengths, the following limitations may have affected our results. Despite these strengths, there are limitations that need to be considered when interpreting study results. We did not have information regarding HPV infections prior to the study start; therefore, participants may have had genital HPV infections prior to enrollment that subsequently cleared. This may have led to misclassification of participants with respect to their classification of their prior genital infection status. It is also possible that participants acquired anal HPV infections prior to the study start. These infections may have been latent and later detected and classified as incident anal infections during the follow-up. Similar to genital HPV infections, it is possible that anal HPV infections may be retained in a latent state in basal

epithelial stem cell pool (185-189). However, re-activation of a latent infection is less likely in immune competent HIM study participants, and the detection of very low viral load of a latent infection is not possible with current HPV DNA detection methods (186-188). Future studies are needed to assess the role of latent HPV infections particularly in immune-competent individuals.

Although 2,285 subjects provided anal specimens at baseline, fewer specimens were available after the fourth visit. Subjects were either lost to follow-up or excluded due to reporting of anal/oral sex with men during follow-up visits. Therefore, we carried out grouped analyses to increase power to assess sequential genital-anal infection for any of the nine types, high-risk types, and low-risk HPV types. Eligible participants may not have reported having male sexual partners due to social desirability. Use of computer assisted techniques for recording sexual behavior history should have minimized the effect of such bias. Misclassification of genital and anal HPV infection status is also possible, especially for infections with low viral load and long time periods between initial infection and assessment of HPV DNA. Given the robust methods used for HPV DNA detection in our study, effect of such misclassification should be minimal (190-194). Furthermore, subjects included in the analyses did not differ from excluded subjects for sexual behavior variables.

In conclusion, men with prior genital HPV infections are at higher risk of acquiring anal infections with the same type, with a more than four-fold increased risk for HPV 16. Auto-inoculation of genital HPV infection along with other unknown factors may explain the presence of anal HPV among MSW. Future studies are needed to assess the role of auto-inoculation and explore other factors associated with high rates of anal HPV infection following a genital infection among MSW. These findings also suggest that the prevention of genital HPV infection

through vaccination and other methods may help curtail anal HPV infection among heterosexual men.

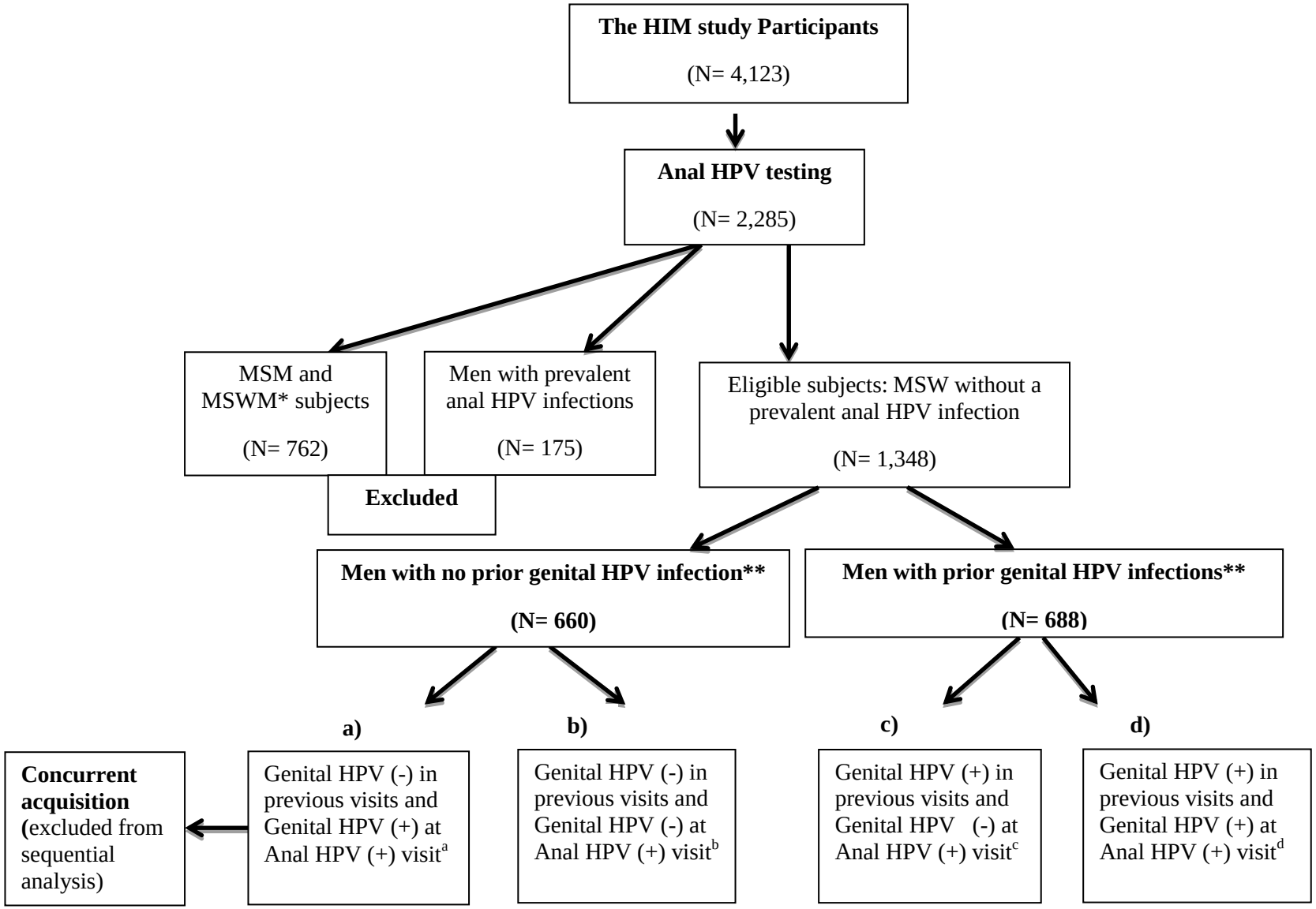

Figure 4.1: Study population for assessment of sequential genital and anal HPV infections among participants of the HIM study

Footnotes: HPV- Human Papillomavirus. Each genital and anal HPV infection assessed is type-specific (genotype concordant). (+) means HPV DNA positive result and (-) means HPV DNA negative result.

*MSM - men who have sex with men, MSWM - Men who have sex with men and women

** No prior genital and prior genital infection status are based on the sequential acquisition of anal HPV infections as indicated by the sub-boxes following each of these groups.

a- Genital and anal infections occurred at the same visit, so cannot assess sequential risk. Number of men positive according to each type: HPV 6- 4, HPV 11- 5, HPV 16- 10, HPV 18 – 3, HPV 31- 1, HPV 33- 1, HPV 45- 8, HPV 52- 4, HPV 58- 2

b- Number of men positive according to each type: HPV 6- 3, HPV 11- 0, HPV 16- 5, HPV 18 – 4, HPV 31- 0, HPV 33- 1, HPV 45- 3, HPV 52- 2, HPV 58- 2

c- Number of men positive according to each type: HPV 6- 1, HPV 11- 0, HPV 16- 3, HPV 18 – 0, HPV 31- 1, HPV 33- 0, HPV 45- 0, HPV 52- 2, HPV 58- 2

d- Previous genital HPV positive visit was used for to assess risk of subsequent anal infections. Number of men positive according to each type: HPV 6- 2, HPV 11- 1, HPV 16- 3, HPV 18 – 0, HPV 31- 0, HPV 33- 0, HPV 45- 0, HPV 52- 3, HPV 58- 2

Table 4.1: Demographic characteristics of MSW (men who have sex with women) according to prior genital HPV infection with any of the nine type HPVs (6, 11, 16, 18, 31, 33, 45, 52, and 58), among subjects anal HPV negative at baseline in the HIM study

	Entire Population (N=1,348)	***No prior genital infection[a] (N=660)***	***Prior genital infection[b] (N=688)***	***P-value[c]***
Country of Residence				
United States	510 (37.8)	265 (52.0)	245 (48.0)	<.0001
Brazil	325 (24.1)	115 (35.4)	210 (64.6)	
Mexico	513 (38.1)	280 (54.6)	233 (45.4)	
Age, years				
18-30	641 (47.6)	313 (48.8)	328 (51.2)	0.8082
31-44	501 (37.2)	242 (48.3)	259 (51.7)	
45-73	206 (15.3)	105 (51.0)	101 (49.0)	
Marital Status				
Single or never married	543 (40.4)	256 (47.1)	287 (52.9)	0.0710
Married/Cohabitating	670 (49.9)	347 (51.8)	323 (48.2)	
Divorced/Separated/Widowed	130 (9.7)	55 (42.3)	75 (57.7)	

Table 4.1 (Continued)

	Entire Population (N=1,348)	*No prior genital infection[a] (N=660)*	*Prior genital infection[b] (N=688)*	*P-value[c]*
Race				
White	589 (44.1)	273 (46.3)	316 (53.7)	<.0001
African Americans	160 (12.0)	61 (38.1)	99 (61.9)	
Asian/Pacific Islander	33 (2.5)	26 (78.8)	7 (21.2)	
Mixed race/Others[d]	555 (41.5)	296 (53.3)	259 (46.7)	
Education, years				
<= 12	578 (43.0)	285 (49.3)	293 (50.7)	0.9708
13-15	363 (27.0)	178 (49.0)	185 (51.0)	
>= 16	404 (30.0)	196 (48.5)	208 (51.5)	
Alcohol use				
No alcohol	276 (20.9)	144 (52.2)	132 (47.8)	0.0010
<0.5 drinks/day	484 (36.6)	258 (53.3)	226 (46.7)	
0.5-2 drinks/day	347 (26.2)	165 (47.6)	182 (52.4)	
> 2 drinks/day	216 (16.3)	83 (38.4)	133 (61.6)	

Table 4.1 (Continued)

	Entire Population (N=1,348)	*No prior genital infection[a] (N=660)*	*Prior genital infection[b] (N=688)*	*P-value[c]*
Smoking status				
Never	776 (57.6)	376 (48.5)	400 (51.5)	0.3432
Former	275 (20.4)	145 (52.7)	130 (47.3)	
Current	296 (22.0)	139 (47.0)	157 (53.0)	
Circumcision				
No	771 (57.2)	377 (48.9)	394 (51.1)	1.0000
Yes	577 (42.8)	283 (49.0)	294 (51.0)	
Lifetime female sexual partners				
0-1	114 (8.5)	79 (69.3)	35 (30.7)	<.0001
2-9	662 (49.1)	358 (54.1)	304 (45.9)	
10-49	484 (35.9)	183 (37.8)	301 (62.2)	
>= 50	88 (6.5)	40 (45.5)	48 (54.5)	
New female sexual partners in previous 6 months				
None	843 (65.0)	440 (52.2)	403 (47.8)	0.0011
1	340 (26.2)	146 (42.9)	194 (57.1)	
>= 2	114 (8.8)	44 (38.6)	70 (61.4)	

Footnotes:

Abbreviation: HPV, human papillomavirus; MSW, men who have sex with women.

a - No prior genital infection group includes participants who are susceptible to acquire anal incident infection and do not have prior type specific genital HPV infection.

b – With prior genital infection group includes participants who are susceptible to acquire anal incident infection and have a prior type specific genital HPV infection.

c - p-values for categorical variables were calculated using the chi-square test with Monte Carlo estimation of p-values, $\alpha = 0.05$,

d - Includes mixed race, American Indians, Alaska natives, Native Hawaiians or other races

Table 4.2: Incidence rate of anal human papillomavirus (HPV) infection, by prior genital HPV infection status among MSW (men who have sex with women) in the HIM Study

No prior genital HPV infection					Prior genital HPV infection				
HPV types	**Subjects at risk**	**Incident infections**	**Person-months**	**Incidence per 1000 person-months (95% CI)**	**Subjects at risk**	**Incident infections**	**Person-month**	**Incidence per 1000 person-months (95% CI)**	**Incidence rate ratio (95% CI)**
Any type*	660	12	5704.0	2.10 (0.12, 3.70)	688	25[a]	5817.8	4.29 (2.90, 6.36)	2.04 (0.99, 4.46)
Low risk types**	1086	3	9542.3	0.31 (0.10, 0.98)	262	4	2288.8	1.75 (0.65, 4.66)	5.56 (0.94, 37.95)
High risk types***	772	11	6695.1	1.64 (0.91, 2.97)	576	21[a]	4895.0	4.30 (2.80, 6.58)	**2.61 (1.20, 6.00)**
HPV 6	1104	3	9554.1	0.31 (0.10, 0.97)	224	3	1967.3	1.52 (0.49, 4.73)	4.86 (0.65, 36.26)
HPV 11	1299	0	11441.5	0.00 (NE)	47	1	437.7	2.28 (0.32,16.22)	NE
HPV 16	1036	5	9034.6	0.55 (0.23, 1.33)	286	6	2415.7	2.48 (1.11, 5.53)	**4.49 (1.14, 18.59)**
HPV 18	1249	4	10902.3	0.37 (0.14, 0.98)	95	0	856.2	0.00 (NE)	NE
HPV 31	1259	0	11164.6	0.00 (NE)	84	1	707.2	1.41 (0.20, 1.00)	NE
HPV 33	1325	1	11691.3	0.09 (0.01, 0.61)	21	0	180.6	0.00 (NE)	NE
HPV 45	1261	3	11102	0.27 (0.09, 0.84)	81	0	682.1	0.00 (NE)	NE
HPV 52	1169	2	10142.3	0.20 (0.24, 1.17)	176	5	1640.3	3.05 (1.10, 6.33)	**15.46 (2.53, 162.33)**
HPV 58	1241	2	10875.8	0.18 (0.08, 0.76)	104	4	950.1	4.21 (1.40, 9.95)	**22.89 (3.28, 253.09)**

Footnotes: Prior genital HPV infection with the same HPV genotype. All the participants were MSWs (men who have sex with women) and never reported having anal or oral sex with men during the 4 year follow-up time.

*Any type includes infections from any of the nine vaccine type HPVs (6, 11, 16, 18, 31, 33, 45 52 and 58)

** Low risk types include HPV 6 and 11

*** High risk types include HPV 16, 18, 31, 33, 45, 52, and 58

NE=Not estimable. Incidence rate ratio and 95% confidence intervals were calculated using a Poisson model with log link function; Included in the 9-valent HPV vaccine.

a- Combining different HPV types for analyses resulted in higher number of incident anal infections among men with prior genital infection with any of the nine types and the high risk HPV types.

Table 4.3: Risk of sequential acquisition of anal human papillomavirus (HPV) infection following a genital HPV infection among MSW (men who have sex with women) in the HIM Study

HPV types	Crude HR (95% CI)	Adjusted HR (95% CI)	Adjusted[d] HR (95% CI)	Adjusted[e] HR (95% CI)	Adjusted[f] HR (95% CI)	Adjusted[g] HR (95% CI)
Any type[*]	**2.19 (1.10, 4.39)**	**2.81 (1.32, 5.99)[a]**	**2.28 (1.10, 4.72)**	**2.53 (1.21, 5.29)**	**5.14 (1.40, 18.82)**	**5.14 (1.40, 18.82)**
Low risk types[**]	**5.41 (1.21, 24.16)**	**5.89 (1.29, 27.01)[b]**	**5.16 (1.15, 23.17)**	**7.80 (1.41, 43.16)**	NE	NE
High risk types[***]	**2.89 (1.38, 6.02)**	**2.65 (1.26, 5.55)[c]**	**3.13 (1.43, 6.83)**	**3.34 (1.53, 7.28)**	**6.95 (1.91, 25.27)**	**6.95 (1.91, 25.27)**
6	4.61 (0.93, 22.84)	NE	4.66 (0.92, 23.56)	**6.19 (1.03, 37.25)**	NE	5.65 (1.13, 28.24)
11	NE	NE	NE	NE	NE	NE
16	**4.63 (1.41, 15.23)**	NE	**4.46 (1.34, 14.85)**	**6.44 (1.91, 21.66)**	**6.50 (1.06, 39.69)**	**5.22 (1.55, 17.57)**
18	NE	NE	NE	NE	NE	NE
31	NE	NE	NE	NE	NE	NE
33	NE	NE	NE	NE	NE	NE
45	NE	NE	NE	NE	NE	NE
52	**15.78 (3.04, 81.92)**	NE	**23.35 (2.57, 211.94)**	**14.52 (2.77, 76.03)**	**17.95 (1.33, 242.47)**	NE
58	**19.60 (3.56, 107.79)**	NE	**23.50 (4.19, 131.84)**	**23.56 (3.72, 149.07)**	**40.54 (4.51, 364.28)**	**35.70 (5.11, 249.28)**

Footnotes:

Reference group consists of men without a prior genital HPV infection of the same genotype

*Any type includes infections from any of the nine vaccine type HPVs (6, 11, 16, 18, 31, 33, 45 52 and 58)

** Low risk types include HPV6 and 11

*** High risk types include HPV 16, 18, 31, 33, 45, 52, and 5

a- Adjusted for marital status and frequency of sexual intercourse with female partners as a time varying covariate based on best fit model

b- Adjusted for country based on best fit model

c- Adjusted for marital status based on best fit model

d- Adjusted for alcohol use as a time varying covariate

e- Adjusted for lifetime female sexual partners as a time varying covariate

f- Adjusted for number of new female partners in past 6-12 months as a time varying covariate

g- Adjusted for frequency of sexual intercourse with female partners as a time varying covariate

CONCLUSION AND RECOMMENDATIONS

HPV infections among men are associated with HPV-related diseases in both men and women. Early studies of HPV focused on infection among women, and it is just recently that we have begun to study HPV infection among men. We have learned that men's response to HPV infection is different from that of women, making it important to study and understand the natural history of HPV infection among men. Results discussed here provide important information in regards to incidence, persistence, and recurrence of anogenital α- mucosal HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58.

Results from section 3.3 suggest that men with anti-HPV antibodies largely remain susceptible to genital HPV infections as these antibodies do not provide protection against subsequent infections with HPV types 6, 11 and 16. There was an indication of a protective effect for persistent HPV 18 infections. Various factors associated with measurement/assessment of serum antibody level can affect anti-HPV antibody levels. With current suboptimal HPV vaccine coverage among both men and women in U.S., it is essential to promote HPV vaccination and other preventive measures to target HPV-associated diseases.

Section 4.3 results provide the first description of the recurrence of genital HPV infections among men. We observed high rates of recurrence for both prevalent (up to 31%) and incident (up to 20%) HPV infections. Recurrence of genital HPV infections was associated with high risk

sexual behaviors, such as greater numbers of new sexual partners and lifetime sexual partners. Analysis of recurrent infections also presents various challenges, including obtaining information about all prior genital infections, issues associated with differentiating infections from steady partners and new partners, and the possibility of HPV latency. Future studies are needed to assess recurrence of HPV infections and explore their role in etiology of HPV-associated diseases.

Results from section 5.3 are also the first to report sequential acquisition of anal HPV infection following a type-specific genital infection among MSW. Men with prior genital HPV 16 infections had a more than four-fold increased risk of acquiring subsequent anal HPV 16 infections. Higher hazard ratios were also observed for any of the nine HPV types, high risk HPV types, and low risk HPV types. In the absence of other explanatory factors, the high risk of sequential genital-anal infection suggests that auto-inoculation of genital infection is a likely mechanism for anal HPV acquisition among MSW. Future studies are needed to further explore this association.

These results provide essential information about the natural history of HPV infection among men. From a public health perspective, it is important to understand the natural history of HPV and use this information to control HPV infections and subsequent diseases in both men and women.

REFERENCES

1. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens — part B: biological agents. Lancet Oncol. 2009;10:321–2.

2. Giuliano AR, Lazcano-Ponce E, Villa LL, Flores R, Salmeron J, Lee JH, et al. (2011). The human papillomavirus infection in men study: human papillomavirus prevalence and type distribution among men residing in Brazil, Mexico, and the United States. Cancer Epidemiol Biomarkers Prev. 2008 Aug;17(8):2036-43.

3. Markowitz LE, Hariri S, Lin C, Dunne EF, Steinau M, McQuillan G and Unger ER (2013). Reduction in Human Papillomavirus (HPV) Prevalence Among Young Women Following HPV Vaccine Introduction in the United States, National Health and Nutrition Examination Surveys, 2003–2010. J Infect Dis. (2013) 208 (3): 385-393

4. Hariri S, Bennett NM, Niccolai LM, Schafer S, Park IU, Bloch KC et al. (2015). Reduction in HPV 16/18-associated high grade cervical lesions following HPV vaccine introduction in the United States - 2008-2012. Vaccine. 2015 Mar 24;33(13):1608-13

5. Nyitray AG, Lin HY, Fulp WJ, Chang M, Menezes L, Lu B, Abrahamsen M, Papenfuss M, Gage C, Galindo CM, Giuliano AR. The role of monogamy and duration of heterosexual relationships in human papillomavirus transmission. J Infect Dis. 2014 Apr 1;209(7):1007-15.

6. Forman D, Marttel CD, Lucey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L, Vignat J, Ferlay J, Bray F, Plummer M, and Franceschi S (2012). Global burden of human papillomavirus and related diseases.Vaccine. 2012 Nov 20;30 Suppl 5:F12-23

7. Human Papillomavirus and Related Diseases Report (2015). Burden of HPV related cancers. ICO information center on HPV and cancer. Retrieved from http://www.hpvcentre.net/statistics/reports/XWX.pdf

8. Chesson HW, Ekwueme DU, Saraiya M, Watson M, Lowy DR, and Markowitz LE (2012). Estimates of the annual direct medical costs of the prevention and treatment of disease associated with human papillomavirus in the United States. Vaccine. 2012 Sep 14;30(42):6016-9

8. Giuliano AR, Lee JH, Fulp W, Villa LL, Lazcano E, Papenfuss MR, Abrahamsen M, Salmeron J, Anic GM, Rollison DE, Smith D. (2011). Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. Lancet. 2011 Mar 12;377(9769).

9. Satterwhite CL, Torrone E, Meites E, et al. Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2008. Sex Transm Dis 2013;40:187–93.

10. Centers of Disease control and Prevention (2014). Human Papillomavirus- Sexually transmitted disease surveillance. Retrieved on 02/2015 from http://www.cdc.gov/std/stats13/other.htm#hpv

11. Division of STD Prevention (1999). Prevention of genital HPV infection and sequelae: report of an external consultants' meeting. Atlanta, GA: Centers for Disease Control and Prevention.

12. Bzhalava D, Guan P, Franceschi S, Dillner J, Clifford G (2013) A systematic review of the prevalence of mucosal and cutaneous human papillomavirus types. Virology 445: 224–231.

13. Bernard HU, Burk RD, Chen Z, Doorslaer K, Hausen H, and Villiers EM (2010). Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 401 (2010) 70–79.

14. Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer. 2003;88(1):63–73.

15. Centers for Disease Control and Prevention (CDC). Human papillomavirus–associated cancers- United States, 2004–2008. MMWR 2012;61(15):258–261

16. Garland SM, Steben M, Sings HL, James M, Lu S, Railkar R, et al. Natural history of genital warts: analysis of the placebo arm of 2 randomized phase III trials of a quadrivalent human papillomavirus (types 6, 11, 16, and 18) vaccine. J Infect Dis. 2009;199(6):805–14.

17. Dunne EF, Nielson CM, Stone KM, Markowitz LE, Giuliano AR. Prevalence of HPV infection among men: a systematic review of the literature. J Infect Dis 2006; 194:1044–57.

18. Howlader N, Noone AM, Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z,Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA SEER Cancer Statistics Review, 1975-2011, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2011/, based on November 2013 SEER data submission, posted to the SEER web site, April 2014.

19. Bezerra AL, Lopes A, Landman G, Alencar GN, Torloni H, Villa LL. Clinicopathologic features and human papillomavirus DNA prevalence of warty and squamous cell carcinoma of the penis. Am J Surg Pathol 2001;25:673–8.

20. Rubin MA, Kleter B, Zhou M, Ayala G, Cubilla AL, Quint WG, et al. Detection and typing of human papillomavirus DNA in penile carcinoma: evidence for multiple independent pathways of penile carcinogenesis. Am J Pathol 2001;159:1211–8.

21. Gross G, Pfister H. Role of human papillomavirus in penile cancer, penile intraepithelial squamous cell neoplasias and in genital warts. Med Microbiol Immunol 2004;193:35–44.

22. Chan PK, Luk AC, Luk TN, Lee KF, Cheung JL, Ho KM, et al. Distribution of human papillomavirus types in anogenital warts of men. J Clin Virol 2009;44:111–4.

23. Skerlev M, Grce M, Sirotkoviae-Skerlev M, Husnjak K, Lipozencic J. Human papillomavirus male genital infections: clinical variations and the significance of DNA typing. Clin Dermatol 2002; 20: 173–8

24. de Martel C1, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, Plummer M. (2012). Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncology, 2012 Jun;13(6):607-15

25. De Vuyst H, Clifford GM, Nascimento MC, et al. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. Int J Cancer 2009;124(7):1626-36.

26. Markowitz LE, Dunne EF, Saraiya M, Chesson HW, Curtis CR, Gee J, Bocchini JA, and Unger ER (2014). Human Papillomavirus Vaccination Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR, August 29, 2014, Vol. 63, No. 5.

27. Petrosky E, Bocchini JA, Hariri S., Chesson H, Curtis CR, Saraiya M, Unger ER, and Markowitz, LE (2015). Use of 9-Valent Human Papillomavirus (HPV) Vaccine: Updated HPV Vaccination Recommendations of the Advisory Committee on Immunization Practices. Morbidity and Mortality Weekly Report (MMWR). Centers for disease control and prevention (CDC). March 27, 2015 / 64(11);300-304

28. Giuliano AR, Palefsky JM, Goldstone S, Moreira ED Jr, Penny ME, Aranda C, et al. Efficacy of quadrivalent HPV vaccine against HPV Infection and disease in males. N Engl J Med 2011;364:401–11.

29. Dessy FJ, Giannini SL, Bougelet CA, Kemp TJ, David MP, Poncelet SM, et al. Correlation between direct ELISA, single epitope-based inhibition ELISA and pseudovirion-based neutralization assay for measuring anti-HPV-16 and anti-HPV-18 antibody response after vaccination with the AS04-adjuvanted HPV-16/18 cervical cancer vaccine. Hum Vaccine 2008;4:425–34.

30. Lu B, Hagensee ME, Lee JH, Wu Y, Stockwell HG, Nielson CM, et al. Epidemiologic factors associated with seropositivity to human papillomavirus type 16 and 18 virus-like particles and risk of subsequent infection in men. Cancer Epidemiol Biomarkers Prev 2010; 19:511–6.

31. Lu B., Viscidi R., Wu Y., Lee H, Nyitray A, Villa L, et al. Prevalent Serum Antibody Is Not a Marker of Immune Protection against Acquisition of Oncogenic HPV16 in Men. Cancer Research, 2012;72(3).

32. Mooij S, Landen A., Van der Klis F., Van der Sande M., et al. No evidence for a protective effect of naturally induced HPV antibodies on subsequent anogenital HPV infection in HIV-negative and HIV-infected MSM. Journal of Infection (2014) 69, 375-386.

33. Wentzensen N, Rodriguez AC, Viscidi R, Herrero R, Hildesheim A, Ghosh A, et al. A competitive serological assay shows naturally acquired immunity to human papillomavirus infections in the Guanacaste natural history study. J Infect Dis 2011;204:94–102.

34. Safaeian M, Porras C, Schiffman M, Rodriguez AC, Wacholder S, Gonzalez P, et al. Epidemiological study of anti-HPV16/18 seropositivity and subsequent risk of HPV16 and -18 infections. J Natl Cancer Inst 2010;102:1653–62.

35. Velicer C, Zhu X, Vuocolo S, Liaw KL, Saah A. Prevalence and incidence of HPV genital infection in women. Sex Transm Dis 2009; 36:696–703.

36. Winer RL, Hughes JP, Feng Q, Xi LF, Cherne S, O'Reilly S, Kiviat NB, Koutsky LA. Early natural history of incident, type-specific human papillomavirus infections in newly sexually active young women. Cancer Epidemiol Biomarkers Prev. 2011 Apr; 20(4):699-707

37. Rositch AF1, Burke AE, Viscidi RP, Silver MI, Chang K, Gravitt PE. Contributions of recent and past sexual partnerships on incident human papillomavirus detection: acquisition and reactivation in older women. Cancer Res. 2012 Dec 1;72(23):6183-90

38. Trottier H1, Ferreira S, Thomann P, Costa MC, Sobrinho JS, Prado JC, Rohan TE, Villa LL, Franco EL. Human papillomavirus infection and reinfection in adult women: the role of sexual activity and natural immunity. Cancer Res. 2010 Nov 1;70(21):8569-77

39. Moscicki AB1, Ma Y, Farhat S, Darragh TM, Pawlita M, Galloway DA, Shiboski S. Redetection of cervical human papillomavirus type 16 (HPV16) in women with a history of HPV16. J Infect Dis. 2013 Aug 1;208(3):403-12.

40. Partridge JM, Hughes JP, Feng Q, et al. Genital human papillomavirus infection in men: incidence and risk factors in a cohort of university students. *J Infect Dis* 2007; 196: 1128‑36.

41. Giuliano AR, Lu B, Nielson CM, et al. Age-specifi c prevalence, incidence, and duration of human papillomavirus infections in a cohort of 290 US men. *J Infect Dis* 2008; 198: 827‑35.

42. Lajous M, Mueller N, Cruz-Valdez A, et al. Determinants of prevalence, acquisition, and persistence of human papillomavirus in healthy Mexican military men. *Cancer Epidemiol Biomarkers Prev* 2005; 14: 1710‑16.

43. Kjaer SK, Munk C, Winther JF, Jorgensen HO, Meijer CJ, van den Brule AJ. Acquisition and persistence of human papillomavirus infection in younger men: a prospective follow-up study among Danish soldiers. *Cancer Epidemiol Biomarkers Prev* 2005**;** 14: 1528‑33.

44. Hernandez BY, McDuffie K, Zhu X, Wilkens LR, Killeen J, Kessel Bet al (2005). Anal human papillomavirus infection in women and its relationship with cervical infection. Cancer Epidemiol Biomarkers Prev. 2005 Nov;14(11 Pt 1):2550-6.

45. Goodman MT, Shvetsov YB, McDuffie K, Wilkens LR, Zhu X, Thompson PJ, et al (2010). Sequential acquisition of human papillomavirus (HPV) infection of the anus and cervix: the Hawaii HPV Cohort Study. J Infect Dis. 2010 May 1;201(9):1331-9. doi: 10.1086/651620.

46. Goodman MT, McDuffie K, Hernandez BY, Wilkens LR, Zhu X, Thompson PJ, et al (2011). The influence of multiple human papillomavirus types on the risk of genotype-concordant incident infections of the anus and cervix: the Hawaii HPV cohort study. J Infect Dis. 2011 Feb 1;203(3):335-40.

47. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr, accessed on day/month/year.

48. Pierce Campbell CM1, Kreimer AR2, Lin HY3, Fulp W3, O'Keefe MT4, Ingles DJ (2015). Long-term persistence of oral human papillomavirus type 16: the HPV Infection in Men (HIM) study. Cancer Prev Res (Phila). 2015 Mar;8(3):190-6

49. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Curado MP, Ferlay J, Franceschi S, Rosenberg PS, Bray F, Gillison ML (2013). Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. J Clin Oncol. 2013 Dec 20;31(36):4550-9.

50. CDC. Human papillomavirus (HPV)-associated cancers. Atlanta, GA: US Department of Health and Human Services, CDC; 2013. Available at http://www.cdc.gov/cancer/hpv/statistics/cases.htm.

51. HPV information center (2014). Human Papillomavirus and Related Cancers, Fact Sheet 2014, USA. ICO information center on HPV and Cancer. Available at www.hpvcentre.net.

52. Jemal A, Simard EP, Dorell C, Noone AM, Markowitz LE, Kohler B, et al. Annual Report to the Nation on the Status of Cancer, 1975–2009, featuring the burden and trends in human papillomavirus (HPV)-associated cancers and HPV vaccination coverage levels. J Natl Cancer Inst 2013;105:175–201

53. American Cancer Society (2015). Cancer Facts and Figures 2015, American Cancer Society, Atlanta, GA. Retrieved from http://www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf

54. Braaten KP, Laufer MR (2008) Human Papillomavirus (HPV), HPV-Related Disease and the HPV Vaccine. Rev Obstet Gynecol 1: 2–10.

55. Bzhalava D, Guan P, Franceschi S, Dillner J, Clifford G (2013) A systematic review of the prevalence of mucosal and cutaneous human papillomavirus types. Virology 445: 224–231.

56. Bernard HU, Burk RD, Chen Z, Doorslaer K, Hausen H, and Villiers EM (2010). Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 401 (2010) 70–79.

57. Villiers EM, Fauquet C, Broker TR, Bernard HU, and Hausena HZ (2004). Classification of papillomaviruses. Virology 324 (2004) 17– 27

58. Koning MN, Quint WGV and Pirog EC (2008). Prevalence of mucosal and cutaneous human papillomaviruses in different histologic subtypes of vulvar carcinoma. Modern Pathology (2008) 21, 334–344.

59. Ho GYF, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med. 1998;338(7);423–8

60. Schiller JT, Day PM, and Kines RC (2010). Current understanding of the mechanism of HPV infection. Gynecol Oncol. 2010 June ; 118(1 Suppl): S12–S17

61. Zheng ZM and Baker CC (2006). Papillomavirus genome structure, expression, and post-transcriptional regulation. Front Biosci. ; 11: 2286–2302

62. Stanley MA (2012). Epithelial Cell Responses to Infection with Human Papillomavirus. Clin Microbiol Rev. 2012 Apr;25(2):215-22

63. Markowitz LE, Dunne EF, Saraiya M, Chesson HW, Curtis CR, Gee J, Bocchini JA, and Unger ER (2014). Human Papillomavirus Vaccination Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR, August 29, 2014, Vol. 63, No. 5.

64. Food and Drug Administration. Product approval-prescribing information. Gardasil [human papillomavirus quadrivalent (types 6, 11, 16, and 18) vaccine, recombinant), Merck & Co, Inc: Food and Drug Administration 2009. Available at http://www.fda.gov/biologicsbloodvaccines/vaccines/approvedproducts/ucm094042.htm

65. Food and Drug Administration. Product approval-prescribing information. Cervarix (human papillomavirus bivalent (types 16 and 18) vaccine, recombinant), GlaxoSmithKline Biologicals: Food and Drug Administration 2009. Available at http://www.fda.gov/biologicsbloodvaccines/vaccines/approvedproducts/ucm186957.

66. Petrosky E, Bocchini JA, Hariri S, Chesson H, Curtis CR, Saraiya M, et al (2015). Use of 9-Valent Human Papillomavirus (HPV) Vaccine: Updated HPV Vaccination Recommendations of the Advisory Committee on Immunization Practices. MMWR Morb Mortal Wkly Rep. 2015 Mar 27;64(11):300-4.

67. Markowitz LE, Dunne EF, Saraiya M, et al.; Centers for Disease Control and Prevention (CDC). Human papillomavirus vaccination: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2014;63(No. RR-05):1–30.

68. Stokley S., Jeyarajah J., Yankey D., Cano M., Gee J., Roark J., et al. Human Papillomavirus Vaccination Coverage Among Adolescents, 2007–2013, and Postlicensure Vaccine Safety Monitoring, 2006–2014 — United States. Centers for Disease Control and Prevention (CDC). Morbidity and Mortality Weekly Report (MMWR). July 25, 2014 / 63(29);620-4

69. Tabrizi SN, Brotherton JM, Kaldor JM, Skinner SR, Cummins E, Liu B., et al. (2012). Fall in Human Papillomavirus Prevalence Following a National Vaccination Program. The Journal of Infectious Diseases 2012;206:1645–51

70. Baldur-Felskov B, Dehlendorff C, Munk C, Kjaer SK. (2014). Early impact of human papillomavirus vaccination on cervical neoplasia--nationwide follow-up of young Danish women. J Natl Cancer Inst. 2014 Mar; 106(3).

71. Gertig DM, Brotherton JM, Budd AC, Drennan K, Chappell G, Saville AM (2013). Impact of a population-based HPV vaccination program on cervical abnormalities: a data linkage study. BMC Med. 2013 Oct 22;11:227.

72. Mariani L, Vici P, Suligoi B, Checcucci-Lisi G, Drury R (2015). Early direct and indirect impact of quadrivalent HPV (4 HPV) vaccine on genital warts: a systematic review. Adv Ther. 2015 Jan;32(1):10-30.

73. Castellsague´ X, Ghaffari A, Daniel RW, Bosch FX, Mun˜oz N, Shah KV. Prevalence of penile human papillomavirus DNA in husbands of women with and without cervical neoplasia: a study in Spain and Colombia. J Infect Dis 1997; 176:353–61.

74. Franceschi S, Castellsague X, Dal Maso L, et al. Prevalence and determinants of human papillomavirus genital infection in men. Br J Cancer 2002; 86:705–11.

75. Svare EI, Kjaer SK, Worm AM, Osterlind A, Meijer CJ, van den Brule AJ. Risk factors for genital HPV DNA in men resemble those found in women: a study of male attendees at a Danish STD clinic. Sex Transm Infect 2002; 78:215–8.

76. Castellsague X, Bosch FX, Munoz N, et al. Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. N Engl J Med 2002; 346:1105–12.

77. Castellsagué X, Bosch FX, Muñoz N, Meijer CJ, Shah KV, de Sanjose S et al (2002). Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. N Engl J Med. 2002 Apr 11;346(15):1105-12.

78. Baldwin SB1, Wallace DR, Papenfuss MR, Abrahamsen M, Vaught LC, Giuliano AR (2004). Condom use and other factors affecting penile human papillomavirus detection in men attending a sexually transmitted disease clinic.Sex Transm Dis. 2004 Oct; 31(10):601-7.

79. Castle PE, Schiff man M, Herrero R, et al. A prospective study of age trends in cervical human papillomavirus acquisition and persistence in Guanacaste, Costa Rica. J Infect Dis 2005;191: 1808–16.

80. Munoz N, Mendez F, Posso H, et al. Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results. J Infect Dis 2004; 190: 2077–87.

81. Giuliano AR, Lu B, Nielson CM, et al. Age-specifi c prevalence, incidence, and duration of human papillomavirus infections in a cohort of 290 US men. J Infect Dis 2008; 198: 827–35.

82. AR Giuliano, E Lazcano, LL Villa, R Flores, J Salmeron, et al (2009). Circumcision and Sexual Behavior: Factors Independently Associated with Human Papillomavirus (HPV) Detection among Men in The HIM Study. Int J Cancer. 2009 Mar 15; 124(6): 1251–1257.

83. Vaccarella S, Lazcano-Ponce E, Castro-Garduno JA, Cruz-Valdez A, Diaz V, Schiavon R, Hernandez P, Kornegay JR, Hernandez-Avila M, Franceschi S. Prevalence and determinants ofhuman papillomavirus infection in men attending vasectomy clinics in Mexico. International journal of cancer. 2006; 119:1934–1939.

84. Svare EI, Kjaer SK, Worm AM, Osterlind A, Meijer CJ, van den Brule AJ. Risk factors for genital HPV DNA in men resemble those found in women: a study of male attendees at a Danish STD clinic. Sex Transm Infect. 2002; 78:215–218.

85. Nielson CM, Harris RB, Dunne EF, Abrahamsen M, Papenfuss MR, Flores R, Markowitz LE, Giuliano AR. Risk factors for anogenital human papillomavirus infection in men. The Journal of infectious diseases. 2007; 196:1137–1145.

86. Ho G Y., Yevgeniy Studentsov, Charles B. Hall, Robert Bierman, Leah Beardsley, Michele Lempa and Robert D. Burk (2002). Risk Factors for Subsequent Cervicovaginal Human Papillomavirus (HPV) Infection and the Protective Role of Antibodies to HPV-16 Virus-Like Particles. The Journal of Infectious Diseases 2002;186:737–42

87. Malik Z., Hailpern SM, and Burk RD (2009). Persistent Antibodies to HPV Virus-Like Particles Following Natural Infection Are Protective Against Subsequent Cervicovaginal Infection with Related and Unrelated HPV. Viral Immunol. 2009 Dec; 22(6): 445–449.

88. Wilson L., Michael Pawlita, Phillip E. Castle, Tim Waterboer, Vikrant Sahasrabuddhe, Patti E. Gravitt, et al (2014). Seroprevalence of 8 Oncogenic Human Papillomavirus Genotypes and Acquired Immunity Against Reinfection. Journal of Infectious Diseases.2014:210 (1 August)

89. Lin SW1, Ghosh A, Porras C, Markt SC, Rodriguez AC, Schiffman M, et al (2013). HPV16 seropositivity and subsequent HPV16 infection risk in a naturally infected population: comparison of serological assays. PLoS One. 2013;8(1):e53067

90. Viscidi, R. P., Mark Schiffman, Allan Hildesheim, Rolando Herrero, Philip E. Castle, Maria C. Bratti, et al (2004). Seroreactivity to Human Papillomavirus (HPV) Types 16, 18, or 31 and Risk of Subsequent HPV Infection: Results from a Population-Based Study in Costa Rica. Cancer Epidemiology, Biomarkers & Prevention. Vol. 13, 324–327, February 2004.

91. Viscidi, R. P., Brad Snyder, Susan Cu-Uvin, Joseph W. Hogan, Barbara Clayman, et al (2005). Human Papillomavirus Capsid Antibody Response to Natural Infection and Risk of Subsequent HPV Infection in HIV-Positive and HIV-Negative Women. Cancer Epidemiology, Biomarkers & Prevention. 2005;14(1). January 2005

92. Trottier H., Silvaneide Ferreira, Patricia Thomann, Maria C. Costa, Joao S. Sobrinho, et al (2010). Human Papillomavirus Infection and Reinfection in Adult Women: the Role of Sexual Activity and Natural Immunity. Cancer Res; 70(21) November 1, 2010

93. Kreimer AR, Alberg AJ, Viscidi R, Gillison ML. Gender differences in sexual biomarkers and behaviors associated with human papillomavirus-16, -18, and -33 seroprevalence. Sex Transm Dis 2004;31: 247–56.

94. Markowitz LE, Sternberg M, Dunne EF, McQuillan G, Unger ER. Seroprevalence of human papillomavirus types 6, 11, 16, and 18 in the United States: National Health and Nutrition Examination Survey 2003-2004. J Infect Dis 2009;200:1059–67.

95. Stone KM, Karem KL, Sternberg MR, McQuillan GM, Poon AD, Unger ER, et al. Seroprevalence of human papillomavirus type 16 infection in the United States. J Infect Dis 2002;186:1396–402.

96. Clifford GM, Shin HR, Oh JK, Waterboer T, Ju YH, Vaccarella S, et al. Serologic response to oncogenic human papillomavirus types in male and female university students in Busan, South Korea. Cancer Epidemiol Biomarkers Prev 2007;16:1874–9.

97. Svare EI, Kjaer SK, Nonnenmacher B, Worm AM, Moi H, Christensen RB, et al. Seroreactivity to human papillomavirus type 16 virus-like particles is lower in high-risk men than in high-risk women. J Infect Dis 1997;176:876–83.

98. Thompson DL, Douglas JM Jr, Foster M, Hagensee ME, Diguiseppi C, Baron AE, et al. Seroepidemiology of infection with human papillomavirus 16, in men and women attending sexually transmitted disease clinics in the United States. J Infect Dis 2004;190:1563–74.

99. Rodríguez AC, Schiffman M, Herrero R, Hildesheim A, Bratti C, Sherman ME, et al (2012). Low risk of type-specific carcinogenic HPV re-appearance with subsequent cervical intraepithelial neoplasia grade 2/3. Int J Cancer. 2012 Oct 15;131(8):1874-81.

100. Moscicki AB, Yifei Ma, Sepideh Farhat, Teresa M. Darragh, Michael Pawlita, Denise A. Galloway, and Stephen Shiboski (2013). Redetection of Cervical Human Papillomavirus Type 16 (HPV16) in Women With a History of HPV16. J Infect Dis. 2013 Aug 1; 208(3): 403–412.

101. Hernandez BY, Ka'opua LS, Scanlan L, Ching JA, Kamemoto LE, Thompson PJ, et al (2013). Cervical and anal human papillomavirus infection in adult women in American Samoa. Asia Pac J Public Health. 2013 Jan;25(1):19-31.

102. Guler T, Uygur D, Uncu M, Yayci E, Atacag T, Bas K, Gunay M, and Yakicier C. (2013). Coexisting anal human papilloma virus infection in heterosexual women with cervical HPV infection. Arch Gynecol Obstet. 2013 Sep;288(3):667-72

103. Nyitray AG, Carvalho da Silva RJ, Baggio ML, Lu B, Smith D, Abrahamsen M, et al (2011). Age-specific prevalence of and risk factors for anal human papillomavirus (HPV) among men who have sex with women and men who have sex with men: the HPV in men (HIM) study. J Infect Dis. 2011 Jan 1;203 (1):49-57.

104. Nyitray AG, Carvalho da Silva RJ, Baggio ML, Smith D, Abrahamsen M, Papenfuss M, et al (2011). Six-month incidence, persistence, and factors associated with persistence of anal human papillomavirus in men: the HPV in men study. J Infect Dis. 2011 Dec 1;204(11):1711-22.

105. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Human papillomaviruses. IARC Monogr Eval Carcinog Risks Hum 2007;90:1–636.

106. Dunne EF, Nielson CM, Stone KM, Markowitz LE, Giuliano AR. Prevalence of HPV infection among men: a systematic review of the literature. J Infect Dis 2006; 194:1044–57.

107. Bezerra AL, Lopes A, Landman G, Alencar GN, Torloni H, Villa LL. Clinicopathologic features and human papillomavirus DNA prevalence of warty and squamous cell carcinoma of the penis. Am J Surg Pathol 2001;25:673–8.

108. Gross G, Pfister H. Role of human papillomavirus in penile cancer, penile intraepithelial squamous cell neoplasias and in genital warts. Med Microbiol Immunol 2004;193:35–44.

109. Chan PK, Luk AC, Luk TN, Lee KF, Cheung JL, Ho KM, et al. Distribution of human papillomavirus types in anogenital warts of men. J Clin Virol 2009;44:111–4.

110. Centers for Disease Control and Prevention (CDC). Human papillomavirus–associated cancers- United States, 2004–2008. MMWR 2012;61(15):258–261

111. Giuliano AR, Palefsky JM, Goldstone S, Moreira ED Jr, Penny ME, Aranda C, et al. Efficacy of quadrivalent HPV vaccine against HPV Infection and disease in males. N Engl J Med 2011;364:401–11.

112. Wentzensen N, Rodriguez AC, Viscidi R, Herrero R, Hildesheim A, Ghosh A, et al. A competitive serological assay shows naturally acquired immunity to human papillomavirus infections in the Guanacaste natural history study. J Infect Dis 2011;204:94–102.

113. Safaeian M, Porras C, Schiffman M, Rodriguez AC, Wacholder S, Gonzalez P, et al. Epidemiological study of anti-HPV16/18 seropositivity and subsequent risk of HPV16 and -18 infections. J Natl Cancer Inst 2010;102:1653–62.

114. Velicer C, Zhu X, Vuocolo S, Liaw KL, Saah A. Prevalence and incidence of HPV genital infection in women. Sex Transm Dis 2009; 36:696–703.

115. Trottier H, Ferreira S, Thomann P, Costa MC, Sobrinho JS, Prado JC, et al. Human papillomavirus infection and reinfection in adult women: the role of sexual activity and natural immunity. Cancer Res. 2010 Nov 1; 70(21):8569-77

116. Viscidi RP, Snyder B, Cu-Uvin S, Hogan JW, Clayman B, Klein RS, et al. Human papillomavirus capsid antibody response to natural infection and risk of subsequent HPV infection in HIV-positive and HIV-negative women. Cancer Epidemiol Biomarkers Prev. 2005 Jan;14(1):283-8.

117. Viscidi RP, Schiffman M, Hildesheim A, Herrero R, Castle PE, Bratti MC, et al. Seroreactivity to human papillomavirus (HPV) types 16, 18, or 31 and risk of subsequent HPV infection: results from a population-based study in Costa Rica. Cancer Epidemiol Biomarkers Prev. 2004 Feb;13(2):324-7

118. Lu B, Hagensee ME, Lee JH, Wu Y, Stockwell HG, Nielson CM, et al. Epidemiologic factors associated with seropositivity to human papillomavirus type 16 and 18 virus-like particles and risk of subsequent infection in men. Cancer Epidemiol Biomarkers Prev 2010; 19:511–6.

119. Lu B, Viscidi R., Wu Y., Lee H, Nyitray A, Villa L, et al. Prevalent Serum Antibody Is Not a Marker of Immune Protection against Acquisition of Oncogenic HPV16 in Men. Cancer Research, 2012;72(3).

120. Mooij S, Landen A., Van der Klis F., Van der Sande M., de Melker HE, Coutinho RA, et al. No evidence for a protective effect of naturally induced HPV antibodies on subsequent anogenital HPV infection in HIV-negative and HIV-infected MSM. Journal of Infection (2014) 69, 375-386.

121. Giuliano AR, Lee JH, Fulp W, Villa LL, Lazcano E, Papenfuss MR, et al. Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. Lancet. 2011 Mar 12; 377(9769):932-40.

122. O'Reilly JM, Hubbard ML, Lessler JT, Biemer PP and Turner CF (1994). Audio and video computer-assisted self-interviewing: Preliminary Tests of New Technologies for Data Collection. J Off Stat. 1994;10 (2):197-214.

123. Adèr, H.J., Mellenbergh, G.J., Hand, D.J. (2008). Advising on Research Methods: A consultant's companion. Johannes van Kessel Publishing. pp. 191–194

124. Le LC, Vu LT. (2012). Audio computer-assisted self-interview compared to traditional interview in an HIV-related behavioral survey in Vietnam. MEDICC Rev. 2012 Oct;14(4):26-31.

125. NIMH Multisite HIV/STD Prevention Trial for African American Couples Group. Designing an audio computer-assisted self-interview (ACASI) system in a multisite trial: a brief report. J Acquir Immune Defic Syndr. 2008 Sep 1;49 Suppl 1:S52-8.

126. Wang SS, Schiffman M, Shields TS, Herrero R, Hildesheim A, Bratti MC, et al. Seroprevalence of human papillomavirus-16, -18, -31, and -45 in a population-based cohort of 10000 women in Costa Rica. Br J Cancer 2003; 89:1248–54.

127. Viscidi RP, Ahdieh-Grant L, Clayman B, Fox K, Massad LS, Cu-Uvin S, et al. Serum immunoglobulin G response to human papillomavirus type 16 virus-like particles in human immunodeficiency virus (HIV)-positive and risk-matched HIV-negative women. J Infect Dis 2003; 187: 194–205.

128. Giuliano AR, Lazcano E, Villa LL, Flores R, Salmeron J, Lee JH, et al. Circumcision and sexual behavior: factors independently associated with human papillomavirus detection among men in the HIM study. IntJ Cancer 2009;124:1251–7.

129. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncol. 2009 Apr; 10(4):321-2

130. Shoultz DA. Human Papillomavirus serum antibodies and their associationwith clinical manifestations of HPV infection in a cohort of sexually active women. Seattle, WA: University of Washington; 1997.

131. Wilson L, Pawlita M, Castle PE, Waterboer T, Sahasrabuddhe V, Gravitt PE, et al. Seroprevalence of 8 oncogenic human papillomavirus genotypes and acquired immunity against reinfection. J Infect Dis. 2014 Aug 1;210(3):448-55

132. Lin SW, Ghosh A, Porras C, Markt SC, Rodriguez AC, Schiffman M., et al. HPV16 seropositivity and subsequent HPV16 infection risk in a naturally infected population: comparison of serological assays. PLoS One. 2013;8(1):e53067.

133. Moscicki AB, Ma Y, Farhat S, Darragh TM, Pawlita M, Galloway DA, et al. Redetection of cervical human papillomavirus type 16 (HPV16) in women with a history of HPV16. J Infect Dis. 2013 Aug 1;208(3):403-12

134. Tong Y, Ermel A, Tu W, Shew M, Brown DR. Association of HPV types 6, 11, 16, and 18 DNA detection and serological response in unvaccinated adolescent women. J Med Virol. 2013 Oct;85(10):1786-93

135. Malik ZA, Hailpern SM, Burk RD. Persistent antibodies to HPV virus-like particles following natural infection are protective against subsequent cervicovaginal infection with related and unrelated HPV. Viral Immunol. 2009 Dec;22(6):445-9

136. Ho GY, Studentsov Y, Hall CB, Bierman R, Beardsley L, Lempa M, et al. Risk factors for subsequent cervicovaginal human papillomavirus (HPV) infection and the protective role of antibodies to HPV-16 virus-like particles. J Infect Dis. 2002 Sep 15;186(6):737-42

137. Theiler RN, Farr SL, Karon JM, Paramsothy P, Viscidi R, Duerr A, et al. High-risk human papillomavirus reactivation in human immunodeficiency virus-infected women: risk factors for cervical viral shedding. Obstet Gynecol 2010; 115:1150–8.

138. Giuliano AR, Palefsky JM, Goldstone S, Moreira ED Jr, Penny ME, Aranda C, et al. Efficacy of quadrivalent HPV vaccine against HPV Infection and disease in males. N Engl J Med 2011;364:401–11.

139. Dessy FJ, Giannini SL, Bougelet CA, Kemp TJ, David MP, Poncelet SM, et al. Correlation between direct ELISA, single epitope-based inhibition ELISA and pseudovirion-based neutralization assay for measuring anti-HPV-16 and anti-HPV-18 antibody response after vaccination with the AS04-adjuvanted HPV-16/18 cervical cancer vaccine. Hum Vaccine 2008;4:425–34.

140. Kreimer AR, Alberg AJ, Viscidi R, Gillison ML. Gender differences in sexual biomarkers and behaviors associated with human papillomavirus16, 18, and 33 seroprevalence. Sex Transm Dis 2004;31:247–56.

141. Markowitz LE, Sternberg M, Dunne EF, McQuillan G, Unger ER. Seroprevalence of human papillomavirus types 6, 11, 16, and 18 in the United States: National Health and Nutrition Examination Survey 2003-2004. J Infect Dis 2009;200:1059–67.

142. Stone KM, Karem KL, Sternberg MR, McQuillan GM, Poon AD, Unger ER, et al. Seroprevalence of human papillomavirus type 16 infection in the United States. J Infect Dis 2002;186:1396–402.

143. Clifford GM, Shin HR, Oh JK, Waterboer T, Ju YH, Vaccarella S, et al. Serologic response to oncogenic human papillomavirus types in male and female university students in Busan, South Korea. Cancer Epidemiol Biomarkers Prev 2007;16:1874–9.

144. Svare EI, Kjaer SK, Nonnenmacher B, Worm AM, Moi H, Christensen RB, et al. Seroreactivity to human papillomavirus type 16 virus-like particles is lower in high-risk men than in high-risk women. J Infect Dis 1997;176:876–83.

145. Thompson DL, Douglas JM Jr, Foster M, Hagensee ME, Diguiseppi C, Baron AE, et al. Seroepidemiology of infection with human papillomavirus 16, in men and women attending sexually transmitted disease clinics in the United States. J Infect Dis 2004;190:1563–74.

146. Giuliano AR, Nyitray AG, Kreimer AR, Pierce Campbell CM, Goodman MT, Sudenga SL, et al. EUROGIN 2014 roadmap: differences in human papillomavirus infection natural history, transmission and human papillomavirus-related cancer incidence by gender and anatomic site of infection. Int J Cancer. 2015 Jun 15;136(12):2752-60.

147. Giuliano AR, Lee JH, Fulp W, Villa LL, Lazcano E, Papenfuss MR, et al. Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. Lancet. 2011 Mar 12; 377(9769):932-40.

148. Giuliano AR, Lazcano-Ponce E, Villa LL, Flores R, Salmeron J, Lee JH, et al. The human papillomavirus infection in men study: human papillomavirus prevalence and type distribution among men residing in Brazil, Mexico, and the United States. Cancer Epidemiol Biomarkers Prev. 2008 Aug;17(8):2036-43.

149. Giuliano AR, Nyitray AG, Kreimer AR, Pierce Campbell CM, Goodman MT, Sudenga SL, et al. EUROGIN 2014 roadmap: differences in human papillomavirus infection natural history, transmission and human papillomavirus-related cancer incidence by gender and anatomic site of infection. Int J Cancer. 2015 Jun 15;136(12):2752-60.

150. Partridge JM, Hughes JP, Feng Q, et al. Genital human papillomavirus infection in men: Incidence and risk factors in a cohort of university students. J Infect Dis 2007;196:1128–36

151. Franceschi S, Castellsague X, Dal Maso L, et al. Prevalence and determinants of human papillomavirus genital infection in men. Br J Cancer 2002;86:705.

152. Smith JS, Gilbert PA, Melendy A, Rana RK, and Pimenta JM. Age-specific prevalence of human papillomavirus infection in males: a global review. J Adolesc Health. 2011 Jun;48(6):540-52.

153. Trottier H, Ferreira S, Thomann P, Costa MC, Sobrinho JS, Prado JC, et al. Human papillomavirus infection and reinfection in adult women: the role of sexual activity and natural immunity. Cancer Res. 2010 Nov 1;70(21):8569-77

154. Winer RL, Hughes JP, Feng Q, Xi LF, Cherne S, O'Reilly S, et al. Early natural history of incident, type-specific human papillomavirus infections in newly sexually active young women. Cancer Epidemiol Biomarkers Prev. 2011 Apr;20(4):699-707

155. Insinga RP, Perez G, Wheeler CM, Koutsky LA, Garland SM, Leodolter S, et al. Incidence, duration, and reappearance of type-specific cervical human papillomavirus infections in young women. Cancer Epidemiol Biomarkers Prev. 2010 Jun;19(6):1585-94.

156. Moscicki AB, Ma Y, Farhat S, Darragh TM, Pawlita M, Galloway DA, et al. Redetection of cervical human papillomavirus type 16 (HPV16) in women with a history of HPV16. J Infect Dis. 2013 Aug 1;208(3):403-12

157. Rodríguez AC, Schiffman M, Herrero R, Hildesheim A, Bratti C, Sherman ME, et al. Low risk of type-specific carcinogenic HPV re-appearance with subsequent cervical intraepithelial neoplasia grade 2/3. Int J Cancer. 2012 Oct 15;131(8):1874-81.

158. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncol. 2009 Apr; 10(4):321-2

159. Joura EA, Giuliano AR, Iversen OE, Bouchard C, Mao C, Mehlsen J, et al. A 9-Valent HPV Vaccine against Infection and Intraepithelial Neoplasia in Women. N Engl J Med. 2015 Feb 19;372(8):711-23

160. Pérez-Gallego L, Moreno-Bueno G, Sarrió D, Suárez A, Gamallo C, and Palacios J. Human papillomavirus-16 E6 variants in cervical squamous intraepithelial lesions from HIV-negative and HIV-positive women. Am J Clin Pathol. 2001 Jul;116(1):143-8.

161. Barzon L, Militello V, Lavezzo E, Franchin E, Peta E, Squarzon L, et al. Human papillomavirus genotyping by 454 next generation sequencing technology. J Clin Virol. 2011 Oct;52(2):93-7.

162. Arroyo LS, Smelov V, Bzhalava D, Eklund C, Hultin E, and Dillner J. Next generation sequencing for human papillomavirus genotyping. J Clin Virol. 2013 Oct;58(2):437-42.

163. Gravitt PE. Evidence and impact of human papillomavirus latency. Open Virol J. 2012;6:198-203.

164. Coutlée F, Rouleau D, Petignat P, Ghattas G, Kornegay JR, Schlag P, et al. Enhanced detection and typing of human papillomavirus (HPV) DNA in anogenital samples with PGMY primers and the Linear array HPV genotyping test. J Clin Microbiol. 2006 Jun;44(6):1998-2006.

165. Stevens MP, Rudland E, Garland SM, and Tabrizi SN. Assessment of MagNA pure LC extraction system for detection of human papillomavirus (HPV) DNA in PreservCyt samples by the Roche AMPLICOR and linear array HPV tests. J Clin Microbiol. 2006 Jul;44(7):2428-33.

166. Gravitt PE, Peyton CL, Apple RJ, and Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol 1998; 36:3020–7.

167. Coutlée, F., D. Rouleau, P. Petignat, G. Ghattas, J. R. Kornegay, P. Schlag, et al. Enhanced detection and typing of human papillomavirus (HPV) DNA in anogenital samples with PGMY primers and the Linear Array HPV genotyping test. J. Clin. Microbiol, 2006. 441998-2006

168. Stevens, M. P., E. Rudland, S. M. Garland, and S. N. Tabrizi. Assessment of MagNA pure LC extraction system for detection of human papillomavirus (HPV) DNA in PreservCyt samples by the Roche Amplicor and Linear Array HPV tests. J. Clin. Microbiol, 2006. 442428-2433

169. Lu B, Viscidi R., Wu Y., Lee H, Nyitray A, Villa L, et al. Prevalent serum antibody is not a marker of immune protection against acquisition of oncogenic HPV16 in men. Cancer Research, 2012;72(3).

170. Rositch AF, Burke AE, Viscidi RP, Silver MI, Chang K, and Gravitt PE. Contributions of recent and past sexual partnerships on incident human papillomavirus detection: acquisition and reactivation in older women. Cancer Res. 2012 Dec 1;72(23):6183-90.

171. Strickler HD, Burk RD, Fazzari M, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. J Natl Cancer Inst 2005; 97: 577-86.

172. Theiler RN, Farr SL, Karon JM, et al. High-risk human papillomavirus reactivation in human immunodeficiency virus-infected women: risk factors for cervical viral shedding. Obstet Gynecol 2010; 115: 1150-8.

173. Nyitray AG, Carvalho da Silva RJ, Baggio ML, Smith D, Abrahamsen M, Papenfuss M, et al (2011). Six-month incidence, persistence, and factors associated with persistence of anal human papillomavirus in men: the HPV in men study. J Infect Dis. 2011 Dec 1;204(11):1711-22.

174. Nyitray AG, Carvalho da Silva RJ, Baggio ML, Lu B, Smith D, Abrahamsen M, et al. Age-specific prevalence of and risk factors for anal human papillomavirus (HPV) among men who have sex with women and men who have sex with men: the HPV in men (HIM) study. J Infect Dis. 2011 Jan 1;203(1):49-57.

175. Nyitray AG, Smith D, Villa L, Lazcano-Ponce E, Abrahamsen M, Papenfuss M, and Giuliano AR. Prevalence of and risk factors for anal human papillomavirus infection in men who have sex with women: a cross-national study. J Infect Dis. 2010 May 15;201(10):1498-508.

176. Goodman MT, McDuffie K, Hernandez BY, Wilkens LR, Zhu X, Thompson PJ, et al. The influence of multiple human papillomavirus types on the risk of genotype-concordant incident infections of the anus and cervix: the Hawaii HPV cohort study. J Infect Dis. 2011 Feb 1;203(3):335-40.

177. Goodman MT, Shvetsov YB, McDuffie K, Wilkens LR, Zhu X, Thompson PJ, et al. Sequential acquisition of human papillomavirus (HPV) infection of the anus and cervix: the Hawaii HPV Cohort Study. J Infect Dis. 2010 May 1;201(9):1331-9

178. Hernandez BY, McDuffie K, Zhu X, Wilkens LR, Killeen J, Kessel B, et al. Anal human papillomavirus infection in women and its relationship with cervical infection. Cancer Epidemiol Biomarkers Prev. 2005 Nov;14(11 Pt 1):2550-6.

179. Hernandez BY, Ka'opua LS, Scanlan L, Ching JA, Kamemoto LE, Thompson PJ, et al. Cervical and anal human papillomavirus infection in adult women in American Samoa. Asia Pac J Public Health. 2013 Jan;25(1):19-31

180. Palefsky JM, Holly EA, Ralston ML, Da Costa M, and Greenblatt RM. Prevalence and risk factors for anal human papillomavirus infection in human immunodeficiency virus (HIV)-positive and high-risk HIV-negative women. J Infect Dis. 2001 Feb 1;183(3):383-91

181. Guler T, Uygur D, Uncu M, Yayci E, Atacag T, Bas K, et al. Coexisting anal human papilloma virus infection in heterosexual women with cervical HPV infection. Arch Gynecol Obstet. 2013 Sep;288(3):667-72.

182. Giuliano AR, Lee JH, Fulp W, Villa LL, Lazcano E, Papenfuss MR, et al. Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. Lancet. 2011 Mar 12; 377(9769):932-40.

183. Giuliano AR, Lazcano-Ponce E, Villa LL, Flores R, Salmeron J, Lee JH, et al. The human papillomavirus infection in men study: human papillomavirus prevalence and type distribution among men residing in Brazil, Mexico, and the United States. Cancer Epidemiol Biomarkers Prev. 2008 Aug;17(8):2036-43.

184. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncol. 2009 Apr; 10(4):321-2.

185. Gravitt PE. Evidence and impact of human papillomavirus latency. Open Virol J. 2012;6:198-203.

186. Lu B, Viscidi R., Wu Y., Lee H, Nyitray A, Villa L, et al. Prevalent serum antibody is not a marker of immune protection against acquisition of oncogenic HPV16 in men. Cancer Research, 2012;72(3).

187. Rositch AF, Burke AE, Viscidi RP, Silver MI, Chang K, and Gravitt PE. Contributions of recent and past sexual partnerships on incident human papillomavirus detection: acquisition and reactivation in older women. Cancer Res. 2012 Dec 1;72(23):6183-90.

188. Strickler HD, Burk RD, Fazzari M, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. J Natl Cancer Inst 2005; 97: 577-86.

189. Theiler RN, Farr SL, Karon JM, et al. High-risk human papillomavirus reactivation in human immunodeficiency virus-infected women: risk factors for cervical viral shedding. Obstet Gynecol 2010; 115: 1150-8.

190. Coutlée F, Rouleau D, Petignat P, Ghattas G, Kornegay JR, Schlag P, et al. Enhanced detection and typing of human papillomavirus (HPV) DNA in anogenital samples with PGMY primers and the Linear array HPV genotyping test. J Clin Microbiol. 2006 Jun;44(6):1998-2006.

191. Stevens MP, Rudland E, Garland SM, and Tabrizi SN. Assessment of MagNA pure LC extraction system for detection of human papillomavirus (HPV) DNA in PreservCyt samples by the Roche AMPLICOR and linear array HPV tests. J Clin Microbiol. 2006 Jul;44(7):2428-33.

192. Gravitt PE, Peyton CL, Apple RJ, and Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol 1998; 36:3020–7.

193. Coutlée, F., D. Rouleau, P. Petignat, G. Ghattas, J. R. Kornegay, P. Schlag, et al. Enhanced detection and typing of human papillomavirus (HPV) DNA in anogenital samples with PGMY primers and the Linear Array HPV genotyping test. J. Clin. Microbiol, 2006. 441998-2006

194. Stevens, M. P., E. Rudland, S. M. Garland, and S. N. Tabrizi. Assessment of MagNA pure LC extraction system for detection of human papillomavirus (HPV) DNA in PreservCyt samples by the Roche Amplicor and Linear Array HPV tests. J. Clin. Microbiol, 2006. 442428-2433

APPENDIX

Appendix A:Table 2.1- Crude and adjusted hazard ratios (HR) according to sero-status for HPV 6, 11, 16 and 18 infections among men in the HIM study

Incident infection	Crude HR and 95% CI	Adjusted HR and 95% CI[a]	Adjusted HR and 95% CI[b]	Adjusted HR and 95% CI[c]	Adjusted HR and 95% CI[d]	Adjusted HR and 95% CI[e]	Adjusted HR and 95% CI[f]
HPV 6							
Sero -	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sero +	1.08 (0.74, 1.56)	1.11 (0.76, 1.62)	1.05 (0.71, 1.55)	1.00 (0.63, 1.57)	1.00 (0.69, 1.46)	0.95 (0.64, 1.40)	0.93 (0.62, 1.39)
HPV 11							
Sero -	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sero +	0.92 (0.55, 1.54)	0.83 (0.49,1.41)	0.88 (0.51, 1.51)	0.78 (0.42, 1.47)	0.83 (0.49,1.39)	0.83 (0.49,1.40)	0.74 (0.43, 1.30)
HPV 16							
Sero -	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sero +	**1.40 (1.08, 1.81)**	**1.36(1.05 1.77)**	**1.39 (1.07 1.82)**	**1.38 (1.03, 1.85)**	**1.32 (1.02, 1.71)**	**1.34 (1.03, 1.76)**	1.31 (0.997, 1.73)
HPV 18							
Sero -	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sero +	0.92 (0.59, 1.43)	0.91 (0.58, 1.43)	1.01 (0.65, 1.57)	0.95 (0.57, 1.59)	0.84 (0.54, 1.31)	0.80 (0.50, 1.23)	0.82 (0.52, 1.30)

Appendix A:Table 2.1 (Continued)

6 month persistent infection	Crude HR and 95% CI	Adjusted HR and 95% CI[a]	Adjusted HR and 95% CI[b]	Adjusted HR and 95% CI[c]	Adjusted HR and 95% CI[d]	Adjusted HR and 95% CI[e]	Adjusted HR and 95% CI[f]
HPV 6							
Sero -	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sero +	0.80 (0.37, 1.73)	0.93 (0.43, 2.00)	0.93 (0.43, 2.01)	0.84 (0.34, 2.07)	0.81 (0.37, 1.75)	0.87 (0.40, 1.89)	0.86 (0.39, 1.87)
HPV 11							
Sero -	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sero +	0.32 (0.08, 1.34)	0.36 (0.09, 1.48)	0.17 (0.02, 1.21)	0.20 (0.03, 1.50)	0.29 (0.07, 1.20)	0.32 (0.07, 1.35)	0.38 (0.09, 1.60)
HPV 16							
Sero -	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sero +	1.26 (0.79, 2.01)	1.27(0.79, 2.03)	1.24 (0.77, 1.99)	1.35 (0.81, 2.27)	1.21 (0.75, 1.93)	1.27 (0.78, 2.06)	1.24 (0.76, 2.04)
HPV 18							
Sero -	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sero +	**0.22 (0.06, 0.91)**	0.24 (0.06, 0.997)	0.24 (0.06, 1.01)	0.17 (0.02, 1.26)	**0.20 (0.05, 0.82)**	**0.23 (0.06, 0.93)**	**0.22 (0.05, 0.92)**

Footnotes: HPV- Human Papillomavirus, HR- Hazard ratios

a - Adjusted for non-sexual behavior covariates

b - Adjusted for lifetime female sexual partners (time varying)

c - Adjusted for new female partners in past 6-12 months (time varying)

d - Adjusted for ever having male oral/anal sexual partners (time varying).

e - Adjusted for lifetime male sexual partners (time varying)

f - Adjusted for new male partners in past 6-12 months (time varying)

Appendix B: Table 2.2 - Crude and adjusted hazard ratios (HR) stratified by sexual orientation for incident HPV 6 and 11 infections among HIM Study participants

	HPV 6				HPV 11			
	Number at risk	Events	Crude HR	Adjusted HR	Number at risk	Events	Crude HR	Adjusted HR
Incident infections								
MSW								
Seronegative	2345	235	1.0	1.0	2374	75	1.0	1.0
Seropositive	170	14	0.86 (0.50, 1.47)	1.06 (0.58, 1.97)[a]	326	6	0.57 (0.25, 1.32)	0.75 (0.32, 1.75)[b]
MSWM								
Seronegative	292	40	1.0	1.0	292	20	1.0	1.0
Seropositive	56	12	1.66 (0.87, 3.16)	1.10 (0.50, 2.46)[c]	96	4	0.62 (0.21, 1.82)	0.60 (0.20, 1.83)[d]
MSM								
Seronegative	77	14	1.0	1.0	77	7	1.0	1.0
Seropositive	22	3	0.81 (0.23, 2.80)	0.50 (0.11, 2.32)[e]	30	3	1.31 (0.33, 5.26)	1.99 (0.44, 8.99)[e]
Six month persistent infections								
MSW								
Seronegative	2345	235	1.0	1.0	2374	75	1.0	1.0
Seropositive	170	14	1.13 (0.49, 2.60)	1.41 (0.56, 3.55)[f]	326	6	0.30 (0.04, 2.20)	0.35 (0.05, 2.57)[g]
MSWM								
Seronegative	292	40	1.0	1.0	292	20	1.0	1.0
Seropositive	56	12	0.47 (0.06, 3.59)	0.53 (0.07, 4.15)[h]	96	4	NE	NE
MSM								
Seronegative	77	14	1.0	1.0	77	7	1.0	1.0
Seropositive	22	3	NE	NE	30	3	1.33 (0.12, 14.72)	1.16 (0.11, 12.83)[i]

Footnotes:

HPV- Human Papillomavirus, HR- Hazard ratios, MSW- Men who have sex with women, MSWM- Men who have sex with women and men, MSM- Men who have sex with men

a- Adjusted for marital status, alcohol use, lifetime female partners (time varying) and new female partners in past 6-12 months(time varying)

b-Adjusted for country, marital status, and new female partners in past 6-12 months(time varying)

c- Adjusted for race, new female partners in past 6-12 months(time varying) and new male partners in past 6-12 months(time varying)

d- Adjusted for marital status, lifetime female partners (time varying) and new male partners in past 6-12 months(time varying)

e - Adjusted for new male partners in past 6-12 months(time varying)

f- Adjusted for alcohol use, new female partners in past 6-12 months (time varying) and frequency of sexual intercourse with female partners in past 6-12 months.

g-Adjusted for smoking, and new female partners in past 6-12 months(time varying)

h- Adjusted for country, i- Adjusted for age

Appendix C: Table 2.3 – Incident rate ratios comparing high (top 33.3%) seropositive to seronegative and low seropositive (lower 66.7%) to seronegative individuals for incident and six month persistent infection for HPV6, 11, 16 and 18.

	High seropositive (Top 33.3%)				Seronegative				
	No.	**At risk**	**Person-years**	**Incidence rate per 100 person-years**	**No.**	**At risk**	**Person-years**	**Incidence rate per 100 person-years**	**Incidence rate ratio and 95% CI**
Incident infections									
HPV 6	12	89	291.8	4.11	319	3105	10898.7	2.93	1.41 (0.72, 2.49)
HPV 11	6	176	628.7	0.95	111	3132	11404.3	0.97	0.98 (0.35, 2.20)
HPV 16	22	137	450.8	4.88	362	2912	10177.2	3.56	1.37 (0.85, 2.11)
HPV 18	7	126	439.6	1.59	200	3202	11578.6	1.73	0.92 (0.37, 1.93)
6-Month persistent infections									
HPV 6	2	89	297.7	0.67	100	3105	11025.7	0.91	0.74 (0.09, 2.75)
HPV 11	0	176	634.4	0.00	37	3132	11451	0.32	0.00 (NE)
HPV 16	5	137	460.2	1.09	120	2912	10310.2	1.16	0.93 (0.30, 2.24)
HPV 18	1	126	442.2	0.23	74	3202	11642.9	0.64	0.36 (0.01, 2.05)
	Low seropositive (Lower 66.7%)				**Seronegative**				
	No.	**At risk**	**Person-years**	**Incidence rate per 100 person-years**	**No.**	**At risk**	**Person-years**	**Incidence rate per 100 person-years**	**Incidence rate ratio and 95% CI**
Incident infections									
HPV 6	18	194	663.75	2.71	319	3105	10898.7	2.93	0.93 (0.54, 1.49)
HPV 11	11	337	1260.1	0.87	111	3132	11404.3	0.97	0.90 (0.44, 1.67)
HPV 16	48	283	964.8	4.98	362	2912	10177.2	3.56	**1.40 (1.01, 1.89)**
HPV 18	15	265	962.6	1.56	200	3202	11578.6	1.73	0.90 (0.49, 1.52)
6-Month persistent infections									
HPV 6	5	194	669.1	0.75	100	3105	11025.7	0.91	0.82 (0.26, 1.99)
HPV 11	2	337	1265.6	0.16	37	3132	11451	0.32	0.49 (0.06, 1.90)
HPV 16	16	283	981.1	1.63	120	2912	10310.2	1.16	1.40 (0.78, 2.37)
HPV 18	1	265	968.7	0.10	74	3202	11642.9	0.64	**0.16 (0.004, 0.93)**

Footnotes: HPV- Human Papillomavirus

Appendix D: Table 4.1a: Risk of sequential acquisition of anal human papillomavirus (HPV) infection following a genital HPV infection

		Country	Age	Marital status	Race
	Crude	Adjusted[a]	Adjusted[b]	Adjusted[c]	Adjusted[d]
HPV types	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Any type*	**2.19 (1.10, 4.39)**	**2.20 (1.09, 4.45)**	**2.25 (1.13, 4.51)**	**2.01 (1.00, 4.05)**	**2.24 (1.11, 4.50)**
Low risk types**	**5.41 (1.21, 24.16)**	**5.90 (1.29, 27.01)**	**5.10 (1.14, 22.83)**	**5.79 (1.29, 25.95)**	**5.56 (1.24, 25.05)**
High risk types**	**2.89 (1.38, 6.02)**	**3.07 (1.46, 6.47)**	**2.94 (1.40, 6.15)**	**2.65 (1.26, 5.55)**	**3.00 (1.42, 6.32)**
6	4.61 (0.93, 22.84)	**5.23 (1.03, 26.59)**	4.41 (0.89, 21.93)	4.66 (0.93, 23.22)	4.69 (0.94, 23.36)
11	NE	NE	NE	NE	NE
16	**4.63 (1.41, 15.23)**	**6.07 (1.82, 20.19)**	**4.66 (1.41, 15.35)**	**4.53 (1.36, 15.04)**	**5.61 (1.67, 18.87)**
18	NE	NE	NE	NE	NE
31	NE	NE	NE	NE	NE
33	NE	NE	NE	NE	NE
45	NE	NE	NE	NE	NE
52	**15.78 (3.04, 81.92)**	**15.20 (2.88, 80.20)**	**18.11 (3.36, 97.11)**	**15.03 (2.88, 78.30)**	**17.93 (3.44, 93.43)**
58	**19.60 (3.56, 107.79)**	**25.12 (4.17, 151.46)**	**20.01 (3.63, 110.89)**	**20.49 (3.69, 113.70)**	**24.84 (3.70, 166.78)**

Footnotes:

Reference group consists of men without a prior genital HPV infection of the same genotype

*Any type includes infections from any of the nine vaccine type HPVs (6, 11, 16, 18, 31, 33, 45 52 and 58)

** Low risk types include HPV6 and 11

*** High risk types include HPV 16, 18, 31, 33, 45, 52, and 5

[a]Adjusted for country

[b]Adjusted for age

[c]Adjusted for marital status

[d] Adjusted for Race

[e]Adjusted for smoking status as a time varying covariate

[f]Adjusted for alcohol use as a time varying covariate

[g]Adjusted for lifetime female sexual partners as a time varying covariate

[h]Adjusted for number of new female partners in past 6-12 months as a time varying covariate

[i]Adjusted for frequency of sexual intercourse with female partners as a time varying covariate

Appendix E: Table 4.1b: Risk of sequential acquisition of anal human papillomavirus (HPV) infection following a genital HPV infection

	Smoking status	Alcohol use	Lifetime female partners	New female partners	Frequency of intercourse
	Adjusted[a]	Adjusted[b]	Adjusted[c]	Adjusted[d]	Adjusted[e]
HPV types	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
All types[*]	**2.14 (1.07, 4.29)**	**2.28 (1.10, 4.72)**	**2.53 (1.21, 5.29)**	**5.14 (1.40, 18.82)**	**5.14 (1.40, 18.82)**
Low risk types[**]	**5.74 (1.28, 25.74)**	**5.16 (1.15, 23.17)**	**7.80 (1.41, 43.16)**	NE	NE
High risk types[***]	**2.83 (1.35, 5.91)**	**3.13 (1.43, 6.83)**	**3.34 (1.53, 7.28)**	**6.95 (1.91, 25.27)**	**6.95 (1.91, 25.27)**
6	4.84 (0.97, 24.14)	4.66 (0.92, 23.56)	**6.19 (1.03, 37.25)**	NE	5.65 (1.13, 28.24)
11	NE	NE	NE	NE	NE
16	**4.21 (1.27, 13.99)**	**4.46 (1.34, 14.85)**	**6.44 (1.91, 21.66)**	**6.50 (1.06, 39.69)**	**5.22 (1.55, 17.57)**
18	NE	NE	NE	NE	NE
31	NE	NE	NE	NE	NE
33	NE	NE	NE	NE	NE
45	NE	NE	NE	NE	NE
52	**15.49 (2.97, 80.79)**	**23.35 (2.57, 211.94)**	**14.52 (2.77, 76.03)**	**17.95 (1.33, 242.47)**	**NE**
58	**25.39 (4.13, 156.24)**	**23.50 (4.19, 131.84)**	**23.56 (3.72, 149.07)**	**40.54 (4.51, 364.28)**	**35.70 (5.11, 249.28)**

Footnotes:

Reference group consists of men without a prior genital HPV infection of the same genotype

*Any type includes infections from any of the nine vaccine type HPVs (6, 11, 16, 18, 31, 33, 45 52 and 58)

** Low risk types include HPV6 and 11

*** High risk types include HPV 16, 18, 31, 33, 45, 52, and 5

aAdjusted for smoking status as a time varying covariate

bAdjusted for alcohol use as a time varying covariate

cAdjusted for lifetime female sexual partners as a time varying covariate

dAdjusted for number of new female partners in past 6-12 months as a time varying covariate

eAdjusted for frequency of sexual intercourse with female partners as a time varying covariate

Appendix F: Institutional Review Board (IRB) approval

USF
UNIVERSITY OF
SOUTH FLORIDA

RESEARCH INTEGRITY AND COMPLIANCE
Institutional Review Boards, FWA No. 00001669
12901 Bruce B. Downs Blvd., MDC035 • Tampa, FL 33612-4799
(813) 974-5638 • FAX(813)974-7091

2/27/2015

Anna Giuliano, Ph.D.
H Lee Moffitt Cancer Center
12902 Magnolia Drive
Tampa, FL 33612

RE: **Expedited Approval for Amendment**
IRB#: Ame12_102660
Title: Natural History of HPV Infection in Men: The HIM Study - MCC# 13930

Dear Dr. Giuliano:

On 2/26/2015, the Institutional Review Board (IRB) reviewed and **APPROVED** your Amendment. The submitted request has been approved for the following:

Revised protocol (Amendment #23.1, 01/12/2015)

Approved Item(s):
Protocol Document(s):
Protocol (Amendment #23.1, 01/12/2015) - clean

We appreciate your dedication to the ethical conduct of human subject research at the University of South Florida and your continued commitment to human research protections. If you have any questions regarding this matter, please call 813-974-5638.

Sincerely,

V Jorgensen MD

E. Verena Jorgensen, M.D., Chairperson
USF Institutional Review Board

Appendix G: Institutional Biosafety Committee (IBC) approval

USF
UNIVERSITY OF
SOUTH FLORIDA

Anna Giuliano, Ph.D.
Interdisciplinary Oncology
MDC 44

3/10/2015

IBC Study number: **1156 Infectious Agent**
IBC Study Title: ***Natural History of HPV Infection in Men: The HIM Study***

Dear Dr.Giuliano:

Your request to renew the above entitled Institutional Biosafety Committee (IBC) Study was reviewed and approved through an expedited process. The IBC acknowledges that this study is currently on going as previously approved. This action will be reported to the IBC at the next regularly scheduled meeting. In addition, please take note of the following:

- **Continuation of the project is approved for a one-year period beginning: 6/1/2015**
- The Principal Investigator shall not modify any research project approved by the IBC until that proposed modification has been registered with and approved by the IBC.
- All operations must be conducted at Biosafety containment level and practices as described in your application and in accordance with the NIH/CDC publication Biosafety in Microbiological and Biomedical Laboratories, 5th Edition.
- Report any significant problems, or any significant research-related accidents and illnesses to Research Integrity and Compliance at 974-0954.
- As a reminder to ensure security of the agent, entry doors to the lab must be shut and locked when the lab is left unattended.
- IBC registration application(s) are approved for a one-year period at the end of which, an annual renewal/amendment application must be submitted for years two (2) and three (3) of the protocol. A new registration application must be reviewed and approved by the Full Committee every three (3) years.

If you have any questions regarding the status of this project, please contact Research Integrity and Compliance at 974-0954.

Sincerely,

BA: dh
cc:DSR

Burt Anderson, Ph.D.
Chairperson
Institutional Biosafety Committee

USF RESEARCH & INNOVATION• RESEARCH INTEGRITY & COMPLIANCE •
INSTITUTIONAL BIOSAFETY COMMITTEE
University of South Florida • 12901 Bruce B. Downs Blvd., MDC35 • Tampa, FL 33612-4799
(813) 974-0954 • FAX (813) 974-7091

Appendix H: Informed consent document:

Subject's Name MCC# 13930
Medical Record # IRB# 102660

Informed Consent to Participate in Research and Authorization to Collect, Use and Share Your Health Information Moffitt Cancer Center/University of South Florida Information to Consider Before Taking Part in this Research Study

Researchers at Moffitt Cancer Center (MCC) study many topics. To do this, we need the help of people who agree to take part in a research study. Research studies include only people who choose to take part. This document is called an informed consent form. Please read this information carefully and take your time making your decision. Ask the researcher or study staff to discuss this consent form with you. Please ask him/her to explain any words or information you do not clearly understand. We encourage you to talk with your family and friends before you decide to take part in this research study. The nature of the study, risks, inconveniences, discomforts, and other important information about the study are listed below.

We are asking you to take part in a research study called: **Natural History of HPV Infection in Men: The HIM Study**

The person who is in charge of this research study is **Anna Giuliano, PhD.** This person is called the **Principal Investigator**. However, other research staff may be involved and can act on behalf of the person in charge.

The research will be conducted at:
H. Lee Moffitt Cancer Center & Research Institute, Inc.
(Moffitt Cancer Center)
12902 Magnolia Drive
Tampa, FL 33612

This research is being sponsored by the National Institutes of Health.

Why is this research being done?

The purpose of this study is to learn about the natural history of Human Papillomavirus (HPV) infection in men. The study will also find out what factors are linked to HPV in men including other sexually transmitted diseases (STDs). If you test positive for syphilis, gonorrhea or chlamydia, we are required by law to report the results to the Florida Department of Health. You will be able to get free medical treatment from the Florida Department of Health for these STDs. You will be given a written report of the results of the STD testing.

HPV is a virus that is transmitted through sexual intercourse. This virus is common in men and women. Some types of this virus can cause warts. Other types of this virus cause changes in the cervix or cancer in women. Anal, penile and oropharyngeal (cancer that develops in the part of the throat just behind the mouth, called the oropharynx) cancers are very rare cancers associated with HPV infections.

Most people with this virus have no signs of infection. There is no effective treatment against the virus yet. In most cases the infection goes away on its own. Little is known about this virus in men. Through this study, the researchers hope to learn more about HPV infection in men, so that they can develop effective programs to reduce HPV disease burden in men and

Medical Record # IRB# 102660
women.
The use of condoms may help lessen the chances of spreading HPV and other STDs between sexual partners. However if the HPV infection is on skin not covered by the condom, it is possible for the disease to spread.

Why are you being asked to take part?

We are asking you to take part in this study because you are a man between 18 and 70 years of age, live in southern Florida, US; Sao Paulo, Brazil; or the state of Morelos, Mexico. A pilot study will be completed consisting of 180 men (about 50 men per year for 3½ years) ages 45 to 70 who live in southern Florida. You have never been told you have penile or anal cancer or genital warts and you are willing to attend scheduled visits every six months in the next 4 years. We want to learn about the natural history of Human Papillomavirus (HPV) infection in men, including what factors are linked to HPV in men.

How long will you be asked to stay in the study?

This study may take up to 5 years to complete; however, you will only be active in this study for approximately 4 years. In order for the researchers to learn about the natural history of HPV, the study will need to last for 4 years.

How often will you need to come for study visits?

A study visit is one you have with the study doctor or study staff. This visit is different than visits with your regular doctor. You will need to come for an additional 9 study visits, once every 6 months for 4 years. Most study visits will take about 45 minutes. Some may be shorter.
At each visit, the doctor or staff will:

• Do an exam of the skin on your genitals. The health provider will swab the outside of your penis and the anal canal with a wet swab to test for HPV. If visible lesions or warts are present, samples will be collected from those areas using a wet swab. You will be told if the health provider finds any sores or lesions that need care.

• Collect an oral cell sample using mouthwash. You will be asked to swish your mouth with mouthwash (this is called an oral rinse). The unused oral sample will be frozen and stored at the Moffitt Cancer Center in Tampa, FL. You will be asked at the end of this consent if you agree to have these samples stored for future testing by the investigators.

• Collect a blood sample. The clinic staff will use a needle to draw 2-3 tablespoons of blood from a vein in your arm. The blood will be tested for HPV antibodies (proteins produced by the body as part of its defense against HPV). Once a year, a portion of this blood will also be tested for HSV 2 (herpes simplex virus 2). The blood sample collected at your last visit will not be tested for HSV2. The blood collected at your second visit will also be tested for syphilis. HSV 2 and syphilis are two sexually transmitted infections. The unused blood will be frozen and stored at the Moffitt Cancer Center in Tampa, FL. You will be asked at the end of this consent if you agree to have

[Original Approval Date: 08/12/2004] Version 24 Revision Date 06/24/2013
Adult Minimal Risk Consent Template – USF Med Rev: 9-3-2010 [MCC Rev: 02-07-2012] Page 2 of 11
Study ID:CR3_102660 Date Approved: 2/1/2015 Expiration Date: 2/1/2016
Subject's Name MCC# 13930
Medical Record # IRB# 102660
these samples stored for future testing by the investigators. Once a year, you will be asked for a urine sample. This urine sample will be tested for chlamydia. You will not provide urine at your

last visit. The urine sample collected at your second visit will also be tested for gonorrhea. Chlamydia and gonorrhea are two sexually transmitted infections.
• Give you a survey to complete. Because HPV is passed on by skin-to-skin contact, such as during sex, you will be asked questions on the survey about your sexual history. For example you may be asked about the number of sex partners you have had, and the date of the last sex. Some of these questions may be personal or sensitive. You can refuse to answer any question.
• Complete a Symptom and Medication Review checklist.
A schedule and sequence of clinic procedures and laboratory specimen collection is attached to this consent form.

How many other people will take part?

About 1000 men, ages 18-44, will take part in this study in the Tampa Bay area. People will also take part at other study sites. A total of about 3000 men will take part. About fifty (50) men per year for the next 3½ years, for a total of 180 men ages 45 – 70, will be added as a pilot study.

Will the treatment you get change if you take part in this study?

The treatment you now get from your regular doctor will not change if you take part in this study. You are free to withdraw from the study at any time without causing bad feelings or changing you medical care.

What other choices do you have if you decide not to take part?

If you decide not to take part in this study, that is okay. This choice will not affect your usual health care. There is no cure for HPV infection in men; however there are options for treating visible warts, however new warts may appear during treatment. Treatment options include; application of therapeutic agents such as bichlor- or trichloracetic acid, podophyllum, or imiquimod, cryotherapy (freezing), laser therapy, or excision (surgical removal).

How do you get started?

If you decide to take part in this study, you will need to sign this consent form. Because the study needs to enroll men who can return for 9 more visits, study staff will ask for your complete contact information. This is needed in order to send reminders about your appointments.
At the end of the visit the study staff will schedule your next clinic visit. You will be given instructions for the next visit: no sex 24 hours before the visit, no washing of genitals with soap the morning of the visit. All these steps are necessary to allow for detection of HPV.
The study staff will contact you to remind you of the next appointment by sending a reminder

letter two weeks prior to the appointment or an e-mail one week before the appointment, whichever is your choice of contact. In addition, the study staff will contact you the day prior to the appointment date. If you wish to withdraw from the study, you should tell the staff so they will not continue to contact you.

What will happen during this study?

At each visit, the health provider will do an exam of the skin on your genitals. The provider will swab the outside of your penis with a wet swab to test for HPV. If visible lesions or warts are present, samples will be collected from those areas using a wet swab. You will be told if the health provider finds any sores or lesions that need care. If during visual physical examination,

genital warts are detected, the clinician can refer you to an outside clinic for treatment and follow up. Prescription may be written for topical cream. If we discover lesions that look like they may be the kind of lesions that could turn into cancer, we will refer you for an evaluation at Moffitt Cancer Center. This evaluation will be free.

You will be asked to give an oral cell sample using mouthwash at each visit. A subset of the study participants (200 men) will also be asked to provide 2 oral samples on the same day for one visit only. You will also be asked to give a blood sample at each visit. The clinic staff will use a clean needle to draw 2-3 tablespoons of blood from a vein in your arm. The blood will be tested for HPV antibodies. Once a year, a portion of this blood will also be tested for HSV 2 (herpes simplex virus 2). The blood collected at your second visit will also be tested for syphilis. Once a year, you will be asked to give a urine sample that will be tested for chlamydia. The urine sample collected at your second visit will also be tested for gonorrhea.

If you have a biopsy of an external genital wart/lesion, you will be asked to give a blood sample during the biopsy visit. The clinic staff will use a clean needle to draw about 1 tablespoon of blood from a vein in your arm. The blood will be used for ADCC (antibody-dependent cellular cytotoxicity) testing. ADCC testing is a new test to test for HPV antibodies (proteins produced by the body as part of its defense against HPV). Even if you are not having a biopsy, we might ask you to donate 1 tablespoon of blood for ADCC testing so that we can compare the blood samples.

You will be given a questionnaire to complete at each visit. Because HPV is passed on by skin-to-skin contact, such as during sex, you will be asked questions on the survey about your sexual history. For example you may be asked about the number of sex partners you have had, and the date you last had sex. Some of these questions may be personal or sensitive. You can refuse to answer any question.

In addition to the questionnaire above, you will be asked to complete a medical history questionnaire, a physical activity questionnaire, a food frequency questionnaire, and a symptom and medication review checklist during your clinic visits. A schedule and sequence of clinic procedures, questionnaires and laboratory specimen collection is attached to this consent form.

Results of HPV testing

The test for human papillomavirus (HPV) being used in this study is a research test and will

include the 37 HPV types most commonly found in the genital area.

• There is no FDA licensed test for HPV detection in men.

• You will receive your results in approximately 6 months.

• You will receive the HPV results from the study clinician, starting at visit 3. The clinician does not have access to the specific HPV type, but you will be told if you are positive or negative for any of the 37 types.

• You will not receive the results from the oral cell sample collection.

Will you be paid for taking part in this study?

We will pay you for the time you volunteer in this study.

• You will get a total of $540 to be part of this study. You will get paid for each completed visit.

• The payment will be as follows: Baseline Visit (Visit 1): $50, Visit 2: $75, Visit: 3: $40, Visit 4: $40, Visit 5: $50, Visit 6: $75, Visit 7: $40, Visit 8: $50, Visit 9: $40, Visit 10: $80.

Will it cost anything to be in this study?

It will not cost you anything to take part in the study.

You will not have to pay for study tests or procedures in this study.

Your insurance plan will not have to pay for any study costs.

What are the potential benefits if you take part in this study?

We don't know if you will get any health benefits by taking part in this study. There may be a benefit to researchers who need to know the natural history of HPV in men to develop methods to prevent infection and to slow or halt its progress to disease. Future studies may help researchers know how HPV is passed on and how long men have the infection. In addition to HPV testing you will also be tested for chlamydia, syphilis, herpes simplex virus 2 and gonorrhea free of charge.

What are the risks if you take part in this study?

The questions you are asked are personal and sensitive and may cause you to feel shame or worry. You can refuse to answer any questions. You can refuse to be part of the study at any time.

When blood is drawn, you may feel some pain and you may faint or feel faint. After blood is collected, you may have a bruise or pain and a slight risk of infection at the site where the blood was taken. Rubbing the penis with a wet swab, and giving a urine sample will not cause you pain, but it may cause some shame or social discomfort. There are no risks to giving an oral cell sample. [Original Approval Date: 08/12/2004] Version 24 Revision Date 06/24/2013

Many people have HPV. Most infections clear on their own. You will not need to make changes in your health care or the health care of your partner. The use of condoms may help lessen the chances of HPV spreading between sexual partners. However if the HPV infection is on skin not covered by the condom, it is possible for the disease to spread. Your female partner(s) should have regular Pap smears regardless of your HPV status.

Risks of undetected HPV in female partners

Some types of the HPV virus that can be transmitted between sexual partners cause changes in the cervix in women. To detect these changes in the cervix, your female partner should get a yearly pap smear. We advise you to talk to your female partner about getting a yearly pap smear no matter what your HPV test results are.

Moffitt Cancer Center Injury Statement

If you believe you have been injured as a result of your participation in this study or if you have questions about your rights as a person who is taking part in a research study, you may call the Moffitt Cancer Center Risk Manager at 813-745-4219. Florida law (Statute 768.28) limits the liability of Moffitt Cancer Center. Moffitt Cancer Center cannot pay for lost wages, disability, or discomfort. A copy of this statute is available upon request at 813-745-1869. This statute provides that damages are available only to the extent that negligent conduct of a Moffitt Cancer Center employee caused your injuries. The damages available in this situation are limited by law.

The Moffitt Cancer Center and investigators have made no provision for monetary compensation in the event of physical illness or injury resulting from this study.
Be aware that your health care payer/insurer might not cover the costs of study-related injuries or illnesses.

The use and disclosure of your personal health information

We understand that information about you and your health is personal, and we are committed to protecting the privacy of that information. Because of this commitment, we must obtain your written authorization before we use or disclose your information for this study.
Research at the Moffitt Cancer Center may be undertaken jointly with the University of South Florida or other persons or entities under an organized health care arrangement. By signing this form you are permitting researchers at Moffitt Cancer Center to use personal health information for research purposes within its organized health care arrangements. You are also allowing the Moffitt Cancer Center to disclose your personal health information to outside organizations or individuals that participate in this study. We may publish what we find out from this study. If we do, we will not let anyone know your name. We will not publish anything that would let people know who you are.
If you do not agree to the use and disclosure described above, you cannot be in the study.

Who will disclose, receive, and/or use your information?

Federal law says we must keep your study records private. We will keep the records of this study private by keeping them in a locked area or on a secure computer. The survey will be marked with a code and not your name. If you give your permission to be contacted, the study staff will not identify the nature of participation. Samples and data sent to the Moffitt Cancer Center and Johns Hopkins University will only be marked with codes. The study staff will keep the codebook that links your name to the code number in a locked file. The consent forms, test results and survey will be kept in locked areas. To do this research, the following people and/or organization(s) will be allowed to disclose, use, and receive your information, but they may only use and disclose the information to the other parties on this list, to you or your personal representative, or as permitted by law:

• Every research site for this study, including the Moffitt Cancer Center, and each site's study team, research staff and medical staff;
• Any person who provides services or oversight responsibilities in connection with this study;
• Every member of the Moffitt Cancer Center workforce who provides services in connection with this study;
• The person who is responsible for the study nationwide or worldwide (study chairperson);
• Any laboratories and other individuals and organizations that use your health information in connection with this study;
• Any sponsor of the study, including the following sponsors: National Institutes of Health
• Any federal, state or local governmental agency that regulates the study (such as the FDA, Florida Department of Health (FDH), the U.S. Department of Health & Human Services (DHHS), and the Office for Human Research Protections (OHRP));
• Other government agencies in this or other countries;

• The designated Protocol Review and Monitoring Committees, Institutional Review Boards, Privacy Boards, Data and Safety Monitoring Board and their related staff that have oversight responsibilities for this study;
• The National Cancer Institute in evaluating the ongoing research of the Moffitt Cancer Center as a Comprehensive Cancer Center.
The organizations and people listed above may employ or pay various consultants and companies to help them understand, analyze and conduct this study. All of these people may not be known now, but if you would like to have more specific information about this at any time during the study, you may ask the study doctor and your questions will be answered.
Moffitt Cancer Center cannot guarantee the privacy of your information, or block further use or distribution, after the information has left the Moffitt Cancer Center. Others listed above may further disclose your information, and may no longer be covered by federal privacy regulations. If all information that does or can identify you is removed from your records, the remaining information will no longer be subject to this authorization and may be used or shared for other purposes.
You might have the right to see and copy your health records related to this research. You

might not be able to see or copy some of your records until after all participants finish the study. If it is necessary for your care, your records will be provided to you or your regular doctor.

What information will be used or disclosed?

By signing below, you authorize the use and disclosure of your entire study record and any medical or other records held by Moffitt Cancer Center, including, but not limited to, HIV/AIDS, mental health, substance abuse or genetic information. The purpose for the uses and disclosures you are authorizing is to conduct the study explained to you during the informed consent and research authorization process and to ensure that the information relating to that study is available to all parties who may need it for research purposes.
Your authorization to use your health information will never expire unless and until you expressly revoke it in writing to the investigator on the first page of this form. If you revoke your authorization, you will not be able to continue in the study.
By signing this form, you authorize the use and/or disclosure of your protected health information described above. Your information may also be used as necessary for your research-related treatment, to collect payment for your research-related treatment (when applicable), and to run the business operations of the Moffitt Cancer Center.
Any data collected prior to your letter will continue to be used as necessary to preserve the integrity of the study, however no additional information will be collected after you withdraw your authorization.

You will receive a signed copy of this form.

What happens if you decide not to take part in this study?

You should only take part in this study if you want to volunteer. You should not feel that there is any pressure to take part in the study, to please the investigator or the research staff. You are free to participate in this research or withdraw at any time. There will be no penalty or loss of benefits you are entitled to receive if you stop taking part in this study.

What if you join the study and then later decide you want to stop?

If you decide you want to stop taking part in the study, tell the study staff as soon as you can.
If for some unplanned reason you move from the area during the study, you agree to tell the study staff as soon as you know you are moving.
It is important to understand that you may leave the study at any time. It is important to let the study staff know if you will not be able to keep an appointment or leave the study.

New information about the study

During the course of this study, we may find more information that could be important to you. This includes information that, once learned, might cause you to change your mind about

being in the study. We will notify you as soon as possible if such information becomes available.

Where can you get the answers to your questions, concerns, or complaints?

If you have any questions, concerns or complaints about this study, or experience an adverse event or unanticipated problem, call Dr. Anna Giuliano at (813) 745-6820.
If you have questions about your rights as a research patient at Moffitt Cancer Center, call the Corporate Compliance Department at The Moffitt Cancer Center at (813) 745-1869.
If you have questions about your rights as a participant in this study, general questions, or have complaints, concerns or issues you want to discuss with someone outside the research, call the USF IRB at (813) 974-5638.

Consent to Take Part in this Research Study and Authorization to Collect, Use and Share Your Health Information

It is up to you to decide whether you want to take part in this study. If you want to take part, please sign the form, if the following statements are true. A representative of the Moffitt Cancer Center must answer your questions completely before providing this form to you. You or your personal representative should read this form and understand it before signing below.

I freely give my consent to take part in this study and authorize that my health information as agreed above, be collected/disclosed in this study. I understand that by signing this form I am agreeing to take part in research. I have received a signed copy of this form to take with me.
Signature of Person Taking Part in Study Date
Printed Name of Person Taking Part in Study

Statement of Person Obtaining Informed Consent / Research Authorization

I attest that the participant named above had enough time to consider this information, had an opportunity to ask questions, and voluntarily agreed to be in this study.
Signature of Person Obtaining Informed Consent / Research Authorization Date
Printed Name of Person Obtaining Informed Consent / Research Authorization

Permission to perform Anal Canal Sampling As part of this study, we would like to test for HPV that may be in your anal canal. In order to get a sample of cells from your anal canal, the clinician will use a swab moistened with normal saline (salt water) to swab the anal canal. The swab will be gently inserted into the anus and turned around while inside the anus.
You do not have to agree to this test to take part in the rest of the study.

Please initial one of the lines below:

____ I agree to anal canal sampling.
____ I DO NOT agree to anal canal sampling.

Permission to store specimens

You are being asked to give permission for your remaining samples to be stored in a specimen bank for future use at the Moffitt Cancer Center and other collaborating institutions. They will be stored without any personal information that could link them to you. The samples will have a code. The study staff will have the codes linking you to the samples stored in a locked, private area. Samples may be used for evaluation of infectious diseases or immune markers of infection. The results of this testing would not be available to you or your physician, and would not alter your care in any manner. If a researcher wishes to use these specimens in a future study, they would need to submit a research proposal to the University of South Florida Human Subjects Committee who will act on your behalf to weigh the risks and benefits of future testing of your specimens, and whether the results can be linked to the coded questionnaire data from this study. You can choose not to have your specimens stored, and still be part of this study. Also, at any time you may change your mind and decide that you do not want to have your samples stored. In that case you must contact Dr. Anna Giuliano at (813) 745-6820 who will ensure that your samples are destroyed.

Please initial one of the lines below:

____ I agree for my remaining samples to be stored and used for future research studies.
____ I DO NOT agree for my remaining samples to be stored and used for future research studies.

Permission to be contacted for future studies
You are being asked if you give your permission to be contacted for future studies conducted by Dr. Giuliano at the Moffitt Cancer Center.

Please initial one of the lines below:

____ I agree to be contacted for future studies.
____ I DO NOT agree to be contacted for future studies.

Subject's Name _____ MCC# 13930
Medical Record # _____ IRB# 102660

Schedule and Sequence of Clinic Procedures and Laboratory Specimen Collection

Visit	1	2	3	4	5	6	7	8	9	10
Month		1	6	12	18	24	30	36	42	48
Event/Test										
Obtain Informed Consent	+									
Study ID # assigned	+									
Full health history & risk factor Questionnaire	+	+								
Symptom & Medication Review	+	+	+	+	+	+	+	+	+	+
Follow-up health history & risk factor Questionnaire			+	+	+	+	+	+	+	+
Medical History Questionnaire	+				+			+		
Physical Activity Questionnaire		+				+				+
Food Frequency Questionnaire*		+				+				
Physical examination of genitalia	+	+	+	+	+	+	+	+	+	+
Oral cell collection using mouthwash	+	+	+	+	+	+	+	+	+	+
Specimen collection using wetted Dacron swab: head, shaft, scrotum, anal canal	+	+	+	+	+	+	+	+	+	+
Swab warts if present	+	+	+	+	+	+	+	+	+	+
Swab lesions if present	+	+	+	+	+	+	+	+	+	+
Biopsy of external genital warts (when applicable)		+	+	+	+	+	+	+	+	+
10 ml blood for ADCC antibody assay (Cases at biopsy visit – controls at the day of the case. 1:1 ratio)										
Serum for syphilis		+								
Serum for HSV		+		+		+		+		
Urine for chlamydia		+		+		+		+		
Urine for gonorrhea		+								
Venous blood draw 10 ml EDTA tube (whole blood)	+					+				+
Venous blood draw 10 ml Red tube (serum)	+	+	+	+	+	+	+	+	+	+

*** This questionnaire will be mailed to your home for you to complete and bring to your next clinic visit.**