ICCR, April 9, 2008: IRB Protocol

Katie Hisert

Duration of Chronicity of HIV Infection as a Predictor of Risk of Malignant or Pre-Malignant Anal Squamous Cell Lesions in HIV+ Men

Study Purpose and Rationale

Anal squamous cell carcinoma (SCC), with an incidence of approximately 5000 cases per year, is a relatively rare malignancy, accounting for less than 2% of all gastrointestinal malignancies. The incidence, however, has been rapidly increasing over the past 30 years. This trend is largely attributable to the increasing prevalence of HIV, particularly in the male homosexual population (men who have sex with men or MSM.) Currently, the rate of anal cancer in the MSM population is at least 35/100,000, which is similar to the rate of cervical cancer in women in the US prior to the introduction of routine pap smears.

It is now widely accepted that the majority of cases of anal SCC occurs secondary to infection of epithelial cells with certain high risk serotypes of Human Papilloma Virus (HPV), which is also the causative agent of most cases of cervical cancer in women. HPV is a small circular double stranded DNA virus. There are over 100 serotypes of HPV, some which are associated with formation of condyloma and others which are associated with malignant transformation of squamous epithelia. Of the approximately 40 serotypes that have been found to infect genital epithelial cells (cervical cells in women, penile cells in men, and anal mucosa in both women and men), 1/4 to 1/3 pose a high risk of inducing malignancy. Worldwide HPV-related cancers make up 5.2% of all malignancies.

The development of HPV-associated anal SCC is thought to occur by the same mechanism as HPV-associated cervical cancer. Viral proteins E6 and E7 interact with host cell tumor suppressors p53 and Rb, respectively. This leads to a malignant transformation of epithelial cells by allowing unchecked cellular cycling. Like cervical cancer, transformation from normal mucosa to invasive cancer occurs in stages that can be identified by both cytology (such as a pap smear), as well as by histology (such as biopsy/colposcopy.) The same nomenclature is used for both cervical cancer and anal cancer. Early lesions detected on cytology are identified as low-grade squamous intraepithelial lesions (LSILs), and are thought to represent HPV replication without evidence of true transformation to malignancy. High grade intraepithelial lesions (HSILs) are thought to represent a true precursor to cancer. Only biopsy can determine if there is an in situ or invasive carcinoma present. Biopsy characterizes lesions as AIN 1 (the true histological equivalent of LSIL, representing HPV replication), AIN 2 (very low preinvasive potential), AIN 3 (true pre-cancerous lesion), or cancer (in situ or invasive).

HPV is the most commonly diagnosed sexually transmitted disease (STD) in the United States. The prevalence of anal infection with HPV is particularly high in MSM, with greater than 60% of HIV negative MSM being infected. Co-infection with HIV is associated with not only a much higher rate of HPV infection (93% of HIV+ MSM are HPV+), but also infection with multiple serotypes of HPV. Studies have shown that 73% of HIV+HPV+ MSM are infected with multiple serotypes of HPV, compared with 23% of HIV-MSM, making HIV+ MSM more likely to be infected with high risk serotypes of HPV. Overall, the relative risk of invasive anal SCC in HIV+ men is 37.9 compared to HIV- individuals. When comparing populations of MSM, HIV+ MSM have a 2 fold increased risk compared with HIV- MSM (an annual incidence of 70/100,000). The incidence of anal SCC in the HIV+ men has increased out proportion to the increase in number of HIV+ patients; according to the CDC website, there were 2.8 cases of anal cancer per 1000 AIDS patients in 1992, but the rate had increased to 24.7 cases per 1000 AIDS patients in 2000.

Several small studies prospective cohort studies have sought to clarify whether immunosuppression (by HIV or in the context of organ transplant or rheumatologic disease) serves as an additional risk for HPV-associated anal SCC. One study monitoring a population of individuals with pre-malignant lesions (AIN3) that were not easily excisable showed that none of the immune competent individual developed anal SCC over the course of the study, but 50% (3 of 6 patients) of the patients who were undergoing immune suppression for inflammatory conditions developed SCC. These data suggested that immune suppression is a further risk factor for progression of pre-malignant lesions to overt cancer. In a separate cross-sectional analysis of HIV+ MSM with HPV infection, the authors correlated patients' HPV serotypes

and levels of HPV DNA with their CD4 counts and found that high levels of DNA representing high risk serotypes was associated with lower CD4 counts. Although not a direct measure of rates of malignancy, these data suggested that HIV+ patient with poorer immune function were at higher risk for anal SCC.

In the mid 1990s, the introduction of highly active anti-retroviral therapy (HAART) meant that HIV+ patients were finally able to suppress HIV viral replication and promote reconstitution of a functional immune system. Consequently, the rates of many AIDS-associated diseases began to rapidly drop off. Morbidity and mortality from opportunistic infections, such as toxoplasmosis and Mycobacterial infections, as well as some oncogenic virus-associated malignancies, such as HHV-8 associated Kaposi's Sarcoma and EBV-associated lymphoma, steadily declined. However, despite the studies suggesting that a weakened immune system was associated with increased risk of anal SCC, rates of HPV-associated premalignant and malignant anal lesions did not decreased in the era of HAART; in fact, it seemed that the incidence of these lesions continued to increase. Prior to HAART (1996), the average annual incidence of invasive anal SCC in men with AIDS was 49/100,00 men, compared with an average annual incidence of 144/100,000 post HAART (1996 and after.)

Thus, new theories have been proposed to account for how HIV infection predisposes HPV infected patients to anal SCC. Several recent studies have postulated that longevity of HIV infection is the true risk factor for development of HPV-associated anal SCC, and that introduction of HAART may in fact increase rates of anal SCC by allowing HIV+ individuals to live longer. There is one retrospective chart review that compared length of HIV-infection in HIV+ patients who had been diagnosed with either AIN or anal SCC. In this study the authors determined that the 7 patients with AIN had been infected with HIV for 5.9 +/- 2.0 years, whereas the 7 patients with anal SCC had been infected with HIV for 12.6 +/- 2.3 years. Thus, although multiple investigators have postulated that increased duration of HIV infection leads to increased risk of HPV-associated anal SCC, further studies need to be performed to evaluate this hypothesis.

Moreover, since it is now clear that recovery of functional immunity does not protect HIV+ HPV+ patients from developing anal SCC, new interventions need to me broadly introduced to reduce morbidity and mortality from this malignancy. Cervical pap smears have drastically reduced the rates of cervical cancer in the United States by 75%. A similar test, the anal pap, can also be performed. Although this procedure is commonly used for studies of anal HPV, AIN, and anal SCC, it is not routinely performed. At least one study has been performed that demonstrated that routine anal cytology screening offers quality-adjusted life expectancy benefits at a cost comparable with other accepted clinical preventive interventions. Thus, if more evidence existed that the risk of developing anal SCC or precancerous lesions increases with duration of HIV-infection, and HIV+ patients are now living longer due to HAART, anal cytology would more likely become standard of care for the aging HIV+ MSM population.

Note: Because HIV and HPV infection are likely to occur in unison in MSM, any association between length of HIV infection and risk of developing HSIL or anal SCC cannot be viewed as a causative association. In all likelihood, duration of HIV infection is a marker for duration of HPV infection, and it is the chronicity of infection with HPV that predisposes individuals to HSIL/anal SCC. However, patients, especially male patients are more likely to be tested for HIV than they are to be tested for HPV, and thus there is more available information about length of infection of patients with HIV.

Study Design and Statistical Analysis

This will be a prospective case control trial to address that hypothesis that increased length of time of infection with HIV correlates with increased risk for the development HSIL or anal SCC in HIV+ MSM.

It is possible to ask a similar question with a retrospective chart review; however, as anal cytology is so rarely performed, the data from old charts are neither sufficient nor necessarily accurate. In an HIV clinic, there be relatively few charts in which HSIL or anal SCC were identified; one would be more likely to find patients with these diagnoses in oncology clinics or surgery clinics (the study noted above comparing duration of infection with HIV and presence of HSIL and SCC was from a surgical department.) Moreover, since anal cytology is not routinely performed, one would want to ensure that the same technique is used for all subjects in the study. Thus, although more costly, a prospective study is a more effective and definitive way to address this hypothesis.

The two study arms will be (a) MSM who have been with HIV for 10 years or more, and (b) MSM who have been infected with HIV+ for less than 10 years. It may be difficult to determine length of infection with HIV for many of the patients, as many patients present to the medical system for the first time with an opportunistic infection, are found to have a CD4 count < 200, and thus have likely been infected for >5 years at the time of presentation. Thus, patients will be separated into short duration and long duration of infection based on the following criteria:

Long term:
-diagnosis of HIV+ more than 10 years ago.
-diagnosis of HIV+ 7-10 years ago, with CD4 <200 at time of diagnosis

Short term:
-diagnosis of HIV within the past 7 years, no opportunistic infections/CD4 count never less than 250
-diagnosis of HIV in past 9 years with known infectious event occurring within 1 yr prior to diagnosis
-diagnosis of HIV within past 9 years with previous earlier HIV test showing HIV negative 10 year ago

There will be likely be potential study subjects whose history precludes them from being categorized in either group based on these definitions. These potential subjects will not be included in the trial.

All potential subjects will undergo anal cytology prior to enrollment. If a potential subject is found to have HSIL or cancer by cytology, he will be excluded from the subject and sent for appropriate follow up. If he is found to have LSIL or normal cytology, he will undergo anoscopy, and any suspicious lesions will be biopsied. Only those with either negative cytology or LSIL on cytology, which is then proved by biopsy to be early (not pre-malignant) AIN, will be randomized. All subjects will receive anal cytology every 6 months. I f a subject is found to have any level of intraepithelial lesion on routine cytologic screening (LSIL or HSIL), the subject will undergo anoscopy and biopsy of suspicious lesions.

Subjects will be followed for a goal time of 24 months. In a previous study of 346 HIV+ MSM (93% HPV+), 20% of subjects who initially had negative cytology and 62% of subjects who had initially had LSIL developed HSIL in 2 years. Overall, 35% of HIV+ subjects were found to have developed HSIL at 2 years. The HIV+ subjects in the previous study represented a mix of newly infected patients and patients who had been infected with HIV for many years. The hypothesis of this study is that patients who have been infected with HIV for longer periods of time have a higher risk of developing premalignant AIN; thus the current hypothesis implies that 35% represents an average of newly infected patients (patient infected for less than 10 years), and those infected for a longer period of time (>10 yrs). This study will be powered to detect a 10% difference in the risk of developing HSIL in 2 years, assuming that the risk in newly infected HIV+ patients is 30%, and the risk is 40% in patients who have been infected for a longer time with HIV.

Using a Chi Square Test to analyze the data, and using an alpha error of 5% (0.05) and a beta error of 80%, calculations demonstrate that at least 376 subjects will be needed in each arm. In order to control for potential drop out or loss to follow up, 400 subjects will ideally be enrolled in each arm.

Power Analysis: $n = 8 * \frac{p_1q_1 + p_2q_2}{\text{effect}^2} + \frac{2}{\text{effect}} + 2$

with $p_1 = 0.30$, $q_1 = 0.70$, $p_2 = 0.40$, $q_2 = 0.60$, and effect = 10% or 0.10, and thus n = 376

Once the data in collected, a Chi squared analysis will be used to determine if there is a significant difference in the rate of developing HSIL or SCC when comparing the two groups of HIV+ patients. A 2x2 table will be constructed to determine the relative risk.

	HSIL or anal SCC	No disease or LSIL
<10 yr HIV+	**a**	**b**
>10 yr HIV+	**c**	**d**

	HSIL or anal SCC	No disease or LSIL	
>10 yr HIV+	**160**	**240**	**400**
<10 yr HIV+	**120**	**280**	**400**

Odds Ratio = ad/bc = 1.56

RR = a*(a+b) / c*(c+d) = 160/120 = 1.33

Study Procedure
Patients will be routinely evaluated at 5 time points over 24 months: 0 months, 6 months, 12 months, 18 months, and 24 months. Evaluations will consist of anal cytology, plus anoscopy with biopsy if anal cytology reveals any grade of intraepithelial lesion. Subjects will be instructed to be aware of pain with defection or rectal bleeding. If these symptoms occur between scheduled evaluations, the subject should contact the study investigators for immediate assessment. The endpoint of the study for each subject will be 24 months of follow up or diagnosis of HSIL or anal SCC at any time during the study.

Examinations will be performed by 1 of 3 trained clinicians. Prior to beginning the study 3 clinicians will be trained to perform the procedures (anal swab and biopsy with anoscope), such that all sampling will be performed in an equivalent manner. Anal cytology and anoscopy will be performed as per a standard, previously described method (see Vadjic, et al. for methods):

“For anal smears a Dacron swab moistened with water is rotated against the anal canal wall for 1 minute before being removed from the anal canal, shaken vigorously in liquid fixative PreservCyt (Cytyc Corporation) transport medium, and then discarded. During the smear the swab is also rotated against the canal wall during removal. For the anoscope biopsy a water lubricated clear plastic disposable anoscope is inserted 3–5 cm into the anal canal and adjusted with the aid of an external light source to reveal the transformation zone before sampling. During withdrawal of the anoscope the distal anal canal is inspected for warts or other abnormality and areas of interest are sampled.

A cytologist will process the samples using the ThinPrep system (Cytyc Corporation) and then an experienced pathologist will perform a conventional cytological assessment of the stained diagnostic cellular material. Anal cytology is classified using the Bethesda criteria for cervical cytology,. The cytologist and pathologist will be blind to sample source”

Any subject who is found to have developed HSIL or anal SCC during the course of the study will be considered to have reached a study “endpoint”, will be discontinued from the study and will directed to appropriate treatment.

Study Drugs
None.

Medical Device
None.

Study Subjects
The study will recruit HIV+ homosexual men (MSM), ages 21-65 from clinics where they routinely receive follow up. All subjects must have a documented positive HIV test. All subjects will be interviewed regarding baseline medical history, sexual practices, tobacco use, alcohol and illicit use, and medication use.

In order to get sufficient numbers of subjects, patients will be recruited from multiple locations, including CUMC in New York City, St. Vincent's Medical Center in New York City, and SF General Hospital in San Francisco. All potential subjects will undergo anal cytology to determine baseline cytology. Subjects who are positive for HSIL or cancer on initial screening will be excluded and directed to appropriate follow up. Subjects with LSIL will undergo anoscopy with biopsy of suspicious lesions. Any biopsy that shows advanced AIN (stage 3) will be an exclusion criterion.

Use of HAART will not be an exclusion criterion, as it is being presumed that number of CD4 T cells and immune status do not play a role in pathogenesis of HPV-associated AIN and anal SCC. Subjects receiving treatment with immunosuppressive agents (such as high dose steroids, anti-B cell therapies or anti-T cell therapies) to treat other medical conditions will be excluded.

As noted above, to be enrolled in this study, subjects need to fall into one of two categories:

Long term:
-diagnosis of HIV+ more than 10 years ago.
-diagnosis of HIV+ 7-10 years ago, with CD4 <200 at time of diagnosis

Short term:
-diagnosis of HIV within the past 7 years, no opportunistic infections/CD4 count never less than 250
-diagnosis of HIV in past 9 years with known infectious event occurring within 1 yr prior to diagnosis
-diagnosis of HIV within past 9 years with previous earlier HIV test showing HIV negative 10 year ago

Recruitment of Subjects
Fliers will be posted at the 3 institutions. Physicians in the HIV clinics will be asked to approach patients whom they think are appropriate.

Confidentiality of Study Data
Study data will be encoded to ensure confidentiality.

Potential Conflict of Interest
None.

Location of the Study
CUMC HP-6 Clinic, New York City
St. Vincent's HIV Center, New York City
San Francisco General Hospital Outpatient HIV Clinic, San Francisco

Potential Risks
There is some possible mild discomfort to the subjects that may be caused by the cytology or anoscopy procedures. As there is no current standard of care for screening for HPV-induced anal pathology, we are not denying subjects any current therapies or care.

Potential Benefits
Individual subjects receive the potential benefit of early identification of pre-cancerous lesions that may be removed or treated prior to these lesions becoming invasive.

The greater benefit of this study is that it will increase the understanding of the natural history of HPV infection and transformation of anal epithelia in HIV+ individuals. If clinicians understand which HIV+HPV+ patients are at greatest risk of developing anal SCC, they will be more alert to which patients need screening and/or evaluation. This study will also hopefully further demonstrate the benefit of using anal cytology to detect pre-malignant lesion in HIV+ MSM, such that treatment can be initiated and invasive cancer can be prevented.

Alternative Therapies
None

Compensation of Subjects
Subjects participating in this study will receive $50 for each follow up session they attend (the enrollment session, 6 months and 12 months.) Subjects will also receive reimbursement for transportation to their evaluation sites.

Cost to Subjects
None.

References

Darragh, T. Anal Cytology for Anal Cancer Screening: Is It Time Yet? *Diagnostic Cytopathology* 2004; 30:371.

Diamond, et al. Increased Incidence of Squamous Cell Anal Cancer Among Men with AIDS in the Era of Highly Active Antiretrovial Therapy. *Sexually Transmitted Diseases* 2005; 32:314.

Fagan, et al. Length of Human Immunodeficiency Virus Disease and Not Immune Status is a Risk Factor for Development of Anal Carcinoma. *American Journal of Surgery* 2005; 190:732.

Goldie, et al. The Clinical Effectiveness and Cost-effectiveness of Screening for Anal Squamous Intraepithelial Lesions in Homosexual and Bisexual HIV-Positive Men. *Journal of the American Medical Association* 1999; 281:1822.

Martin F and Baker M. Anal Intraepithelial Neoplasia in HIV Positive People. *Sexually Transmitted Infections* 2001; 77:327.

Matthews, W. *Screening for Anal Dysplasia Associate with Human Papillomavirus*. Topics in HIV Medicine 2003; 11:45.

Palefsky, et al. Prevalence and Risk Factors for Human Papillomavirus Infection of the Anal Canal in Human Immunodeficiency Virus (HIV) – Positive and HIV-Negative Homosexual Men. *Journal of Infectious Diseases* 1998; 177:361.

Palefsky, et al. Virologic, Immunologic, and Clinical Parameters in the Incidence and Progression of Anal Squamous Intraepithelial Lesions in HIV- Positive and HIV-Negative Homosexual Men. *J Acquired Immune Deficiency Syndrome* 1998; 17:314.

Palefsky, et al. Anal Squamous Intraepithelial Lesions in HIV-Positive and HIV-Negative Homosexual and Bisexual Men: Prevalence and Risk Factors. *J Acquired Immune Deficiency Syndrome* 1998; 17:320.

Palefsky, Joel. Human Papillomavirus-Related Tumors in HIV. *Current Opinion in Oncology* 2006; 18:463.

Piketty, et al. High Prevalence of Anal Squamous Intraepithelial Lesions in HIV-Positive Men Despite the Use of Antiretroviral Therapy. *Sexually Transmitted Diseases* 2004; 31:96.

Scholefield, et al. Malignant Transformation of High-Grade Anal Intraepithelial Neoplasia. *British Journal of Surgery* 2005; 92:1133.

Vajdic, et al. Blind sampling is superior to anoscope guided sampling for screening for anal intraepithelial neoplasia. *Sexually Transmitted Infections* 2001; 81:415.