WOMEN'S INTERAGENCY HIV STUDY
SECTION 22: ANAL SUBSTUDY PROTOCOL

A. HYPOTHESES

1. Risk factors for anal HPV infection in women include receptive anal intercourse, smoking, cervical HPV infection, HIV positivity, low CD4 level and high HIV viral load. The effect of HAART on detection of anal HPV infection, the number of anal HPV types and level of HPV infection will be most pronounced among women who initiate HAART at an early stage in the natural history of their HIV infection, i.e., those with a high CD4 nadir.

2. Risk factors for prevalent ASIL and ASIL progression include HIV positivity, low CD4 level, high HIV viral load, infection with multiple anal HPV types, cervical HPV infection, smoking and medical conditions such as anal fissure or fistula. HAART will have its most beneficial effect on the natural history of ASIL among those women who initiate HAART at an early stage in the natural history of their HIV infection.

3. Site-specific differences between the cervix and anus determine the course of HPV infection and HPV-associated lesions at these sites. Strain variants will be the same in the cervix and anus indicating a possible common exposure at the two sites or spread from one site to the other. Conversely, different variants at each site could indicate separate exposures, or less likely, site-specific variant tropism.

4. Development of anal HSIL is linked to gene amplification on the long arm of chromosome 3. Women who have concomitant anal and cervical HSIL will show the same genetic changes in their anal and cervical lesions suggesting a single common molecular pathway to development of anal and cervical cancer in these patients.

B. SCIENTIFIC AIMS

1. Study the natural history of anal HPV infection among HIV+ and high risk HIV- women in the highly active antiretroviral therapy (HAART) era.

2. Study the development and progression of ASIL among HIV+ and high risk HIV- women in the HAART era.

3. Compare the natural history and risk factors for anal HPV infection and cytologic abnormalities to those of cervical HPV infection and cytologic abnormalities among HIV+ and high risk HIV- women. Strain variants of HPV 16, 18 and 31 will be compared in the anus and cervix.

4. Analyze genetic changes in ASIL in women and compare these to CSIL in the same subjects.

C. BACKGROUND

Rationale for studying anal HPV infection and ASIL in the WIHS cohort

We will perform a study of the natural history of anal squamous intraepithelial lesions (ASIL) and anal HPV infection in HIV+ and high-risk HIV- women. The rationale for this study is:

1. Anal cancer is increasing in the United States among both men and women. In the general population, anal cancer is about twice as common in women as it is in men.[1] Based on U.S. data from the Surveillance, Epidemiology, and End Results (SEER) program for 1973-1989 and from the Connecticut Tumor Registry for 1940-1988, since 1960 the incidence of anal cancer in Connecticut increased 1.9-fold among men and 2.3-fold among women. The incidence was

lowest among white men (1973-1989 average: 0.41/100,000) and highest among African-American women (1973-1989 average: 0.74/100,000).[2] In Denmark, using a 50-year old national cancer registry, Melbye, et al., reported that between 1957 and 1987, the incidence of anal cancer among Danish women more than tripled to 7.4/1,000,000.

The incidence of anal cancer is highest among men with a history of receptive anal intercourse and is estimated to be as high as 36/100,000. Therefore it is approximately five times the incidence of cervical cancer in this country.[3] In recent years, the incidence was found to be approximately two-fold higher than that among HIV+ men who have sex with men (MSM).[4] Recently, data obtained from the U.S. AIDS-Cancer registry match show that HIV+ women were at a 6.8-fold higher risk of developing anal cancer than women in the general population.[5]

2. In studies of the natural history of anal HPV infection and ASIL prior to the era of highly active antiretroviral therapy (HAART) among MSM, we found that essentially all HIV+ men and most HIV- MSM had detectable anal HPV infection. The incidence of the anal cancer precursor, high grade ASIL (HSIL), was extraordinarily high in both HIV+ and HIV- men but was higher among HIV+ men.[6, 7, 8, 9]

3. Little is known about the effect of HAART on the natural history of anogenital HPV infection. In one small study, there was evidence that some women with CSIL improved after beginning HAART.[10] Ahdieh and Minkoff, et al., reported at the Barcelona International Papillomavirus Conference that women on HAART were more likely statistically to show regression of cervical cytology, but as in the Heard study, the majority of women on HAART did not regress.[11] In another study presented at the AIDS Conference in Durban, a group from Italy showed no regression of cervical disease among women on HAART.[12] Consistent with these data we have seen little regression of HSIL among MSM who have had undetectable HIV viral load due to HAART and increased CD4 levels, and we continue to see high rates of progression from LSIL to HSIL among these men.

   It is known that progression of cervical HSIL to invasive cervical cancer may take up to 20 years in some cases.[13] Based on this observation, our ASIL natural history studies and the increased survival time among MSM on HAART, we project a further <u>increase</u> in the incidence of anal cancer, because HIV+ individuals may now have ample time for HSIL to progress to invasive anal cancer. We believe that we have already begun to see this increase with the recent demonstration that the incidence of anal cancer among HIV+ MSM is about twice that of HIV- MSM.

4. Based on the above, we performed a pilot prospective study of anal HPV infection and ASIL in HIV+ and HIV- women at the San Francisco WIHS site. The data show a high prevalence of anal HPV infection[14] and ASIL in this group,[15] comparable to our studies in MSM when adjusted for CD4 level. We now propose to extend this pilot study to include three WIHS sites to provide us with sufficient numbers of subjects and statistical power to perform multivariate analysis of risk factors for ASIL prevalence and disease progression. Among the key questions that can only be addressed in a study as large as the WIHS are: Other than HIV positivity, CD4 level and HIV viral load, what are the behavioral and biological risk factors for anal HPV infection and ASIL? For progression to anal HSIL? What is the relationship between cervical and anal HPV infection, and between cervical and anal SIL? Are the HPV strain variants the same at the two mucosal sites? What genetic changes occur in ASIL? What are the specific genes that are deleted or overexpressed and are these the same changes as those found in CSIL?

D. PRELIMINARY STUDIES

Prevalence of anal HPV infection and ASIL in HIV+ and HIV- women

Based on our findings in the MSM we performed a prospective pilot study of anal HPV infection and ASIL at the San Francisco WIHS site.[14, 15] Our goals were to study a larger population than the earlier studies, obtain prospective data to establish the feasibility of performing anal studies in the WIHS with the eventual goal of studying the entire WIHS population. To perform the study, 359 women participating in the WIHS study at the San Francisco site were asked to participate in the anal HPV study. Of these women, 251 HIV+ and 68 HIV- women (89%) agreed to participate. A supplemental questionnaire specific to anal HPV infection and ASIL was administered after the routine WIHS questionnaire. After samples were taken for cervical cytology and HPV testing, two anal samples were obtained for anal cytology and anal HPV testing. Women diagnosed with abnormal anal cytology were contacted to return for a separate visit for high-resolution anoscopy (HRA). These procedures were repeated every six months at the routine WIHS study visits for up to three years, unless the study endpoint, diagnosis of anal HSIL, was reached. Women were then referred out of the anal study for further evaluation and treatment. A high proportion of the HIV+ women (76%) and HIV- women (75%) reached the study endpoint. Compliance with returning for HRA was good but depended on the degree of severity of the anal cytology. Overall, 77% of HIV+ women with LSIL on anal cytology presented for anoscopy, whereas 90% of women with HSIL on cytology appeared for anoscopy.

The results of the baseline studies are as follows: 280 women had a sample that was interpretable by PCR. HPV DNA was detected in the anal samples from 170 of 223 (76%) HIV+ women and 24 of 57(42%) HIV- women ($p<.001$). Type 16 was found most frequently in HIV+ women. This was followed by types 58, 53, 61, 70, Pap155, 18, and Pap291 that were found in at least 5% of the HPV positive samples. Anal samples were available for HC testing for 242 HIV+ and 67 HIV- women. Using this test, HPV was detected in 182 (75%) HIV+ women, and 20 (30%) HIV- women ($p<.001$).

HIV+ women had an increased risk of anal HPV infection as detected by PCR (RR=1.8, 95% CI 1.3-2.5), or as detected by HC (RR=2.5, 95% CI 1.7-3.7). Among HIV+ women, HPV infection increased as CD4 counts decreased using either method ($p=.04$ for PCR, $p=.001$ for HC). However, there was no association between HIV viral load and anal HPV infection. The mean number of specific HPV types detected by PCR ($p = .0004$) and mean HC RLU ratio ($p = .0001$) were higher in HIV+ women than HIV- women. Among HIV+ women with HPV infection, the mean number of specific HPV types detected by PCR (p for linear trend = .02) and the mean HC RLU ratio (p for linear trend = .0001) increased with decreasing CD4 levels. There was no apparent association between HIV viral load and any of these measures of the amount of HPV infection.

Lifetime and recent exposure to potential risk factors for detection of anal HPV DNA were evaluated in HIV+ women. Potential risk factors were not evaluated in HIV- women because of small numbers. In univariate analysis, risk factors for anal HPV infection among HIV+ women included younger age (p for trend = .05), race (African-American vs. white, non-hispanic (RR .82, 95% CI .72-.94), other vs. white, non-hispanic (RR .68, 95% CI .51-.92) and lifetime history of an AIDS diagnosis not based on CD4 level. A number of other factors were associated with increased risk of HPV detection in univariate analysis but only age, race and lifetime use of AZT remained significant after adjustment for CD4 level. History of anal intercourse was not a risk factor for detection of anal HPV infection.

Anal cytology was missing or insufficient at baseline for 16 HIV+ women and 7 HIV- women. Overall 26% of HIV+ women had abnormal anal cytology compared to 8% of HIV- women. Of

the 66 women with anal cytology showing ASCUS, LSIL, or HSIL, 46 (70%) returned within six months for an anal exam including colposcopy and biopsy of visible anal lesions. Although few cases of HSIL were found on cytology, 30% of women with abnormal anal cytology were found to have anal HSIL on biopsy demonstrating the need to follow abnormal cytology results with histologic assessment.

HIV+ women were significantly more likely to have abnormal anal cytology than HIV- women (RR=3.2, CI: 1.3-7.5). When stratified by CD4 count, HIV+ women were at increased risk of abnormal anal cytology as CD4 level decreased (test for trend, p<.0001). HIV+ women also were at increased risk of abnormal anal cytology as HIV viral load increased (test for trend p=.02). HIV+ women with abnormal cervical cytology at the same visit were at increased risk of abnormal anal cytology compared to HIV+ women with normal cervical cytology (RR=2.2, CI: 1.4-3.3). Too few HIV- women had abnormal anal cytology for evaluation of association with cervical cytology or other risk factors.

Abnormal anal cytology was associated with having anal HPV infection by PCR (RR 4.0, 95% CI 4.0, 1.5-10.5) or HC (RR 4.3, 95% CI 4.0, 1.6-11.4). HPV DNA quantity as measured by the HC relative light unit ratio also correlated with abnormal anal cytology (trend p<.0001). Risk factors associated with abnormal anal cytology were adjusted for CD4 level and HPV positivity using HC. Several factors remained significant after adjustment including history of anal intercourse, history of major chronic illness and history of diarrhea for greater than one month. There were insufficient HIV- women in the study to perform this analysis. Our data suggest that history of anal intercourse and factors that may be associated with anal irritation may be associated with abnormal anal cytology, as are HIV positivity, anal HPV infection and lower CD4 level.

E. METHODS

1. OVERVIEW

   a. Each subject will have an interview detailing behavioral and medical health issues as part of the WIHS study. Subjects in the anal study will also undergo a short supplemental interview more specific to issues of anal disease.

   b. The external anus will then be examined for the presence of visible warts, hemorrhoid, discharge or ulcerations. Lesions such as ulcers or warts are recorded on the lesion form. After the clinician changes gloves, he or she will insert a moistened Dacron swab to the distal rectum for collection of cells for anal cytology. The swab will be inserted into a ThinPrep vial for anal cytology and anal HPV testing.

   c. Women with abnormal anal cytology will be referred for high resolution anoscopy (HRA) and biopsy of visible disease. She will return at a separate time for this purpose. If a participant has a history of disease of the heart valves requiring antibiotics for procedures such as teeth cleaning, she will be given an antibiotic such as amoxicillin one hour before the procedure. If she has an allergy to antibiotics such as penicillin, she will be asked to tell the doctor or nurse and another antibiotic regimen recommended by the American Heart Association will be given.

   d. At HRA an anoscope is then inserted to the distal rectum. A wooden stick wrapped in gauze soaked in 3% acetic acid is inserted and the anoscope removed to allow the acetic acid to come into contact with the mucosa. After one minute, the stick is removed and the anoscope reinserted. The location of areas suspicious for HPV-related disease will be recorded on a standard form. Among the signs of HPV-associated disease that will be sought are leukoplakia, vascular punctation, and papillary topography. These areas will then be biopsied and the tissue fixed in formalin.

e. Drawings, photographs or computer images may be used to record the appearance of the anal canal.

f. For our genetic analyses, in most cases the standard formalin-fixed biopsy suffices. In some cases, particularly if the lesion is large, we will obtain a second fresh-frozen biopsy for genetic analysis.

g. Subjects will be examined in this fashion every six months unless the study endpoint, i.e., diagnosis of anal HSIL on cytology or biopsy, is reached. At that time, she will be withdrawn from the study and referred back to her primary care provider and given a referral to an anal surgeon. Further clinical care, especially if the woman declines therapy, is coordinated between the women's primary care clinician and the surgeon.

2. STUDY POPULATION

WIHS participants who are participants at the UCSF, Brooklyn and Chicago sites, and who have no history of anal high-grade lesions or anal cancer. Both original study participants and newly recruited participants will be recruited to the anal study. There are no specific goals for the numbers of women in each group but we are aiming to have approximately 50% existing and 50% new participants.

3. SAMPLE SIZE

We will study 650 women at the San Francisco, Chicago and Brooklyn sites every six months with anal cytology and anal HPV. Women with abnormal anal cytology will be referred for high-resolution anoscopy and biopsy of visible lesions. For HIV+ women, we will not use the baseline data collected from the women in the San Francisco cohort if they had been enrolled in the pilot study. Assuming that one third of the women are from San Francisco we will have 333 HIV+ women from the other two centers available for analysis of prevalence data. We will perform separate analyses on the HAART and non-HAART users as well as in combination. We assumed that 33-67% would be on HAART. Therefore, the smaller group would have at least 111 women and the larger would have at most 222 women. Table 1, below, shows sample size calculations for prevalent disease. For HIV- women we will combine the baseline data collected in the present study from the non-San Francisco women with the pilot study data collected from the San Francisco women. This will provide 168 HIV- women for analysis.

For the follow-up analyses we will use data collected on all three cohorts so the sample sizes for various analyses will be 500 HIV+ women and 150 HIV- women (Table 2).

Table 1. Sample Size Calculations for Prevalence (modified to include n=140)

% with risk factor	Probability of disease, risk factor absent (%)	Probability of disease, risk factor present (%)	RR*	Power % n=111	Power % n=222	Power % n=333
10	10	40	4	50	88	98
		30	3	21	51	74
	20	60	3	76	97	99
	40	80	2	81	97	99
	60	90	1.5	72	92	98
25	5	15	3	22	49	70
	10	30	3	57	89	98
		20	2	18	38	55
	20	40	2	47	79	93
	40	60	1.5	44	74	89
	60	90	1.5	91	99	99
50	5	20	4	67	92	98
		15	3	41	70	86
	10	30	3	75	96	99
		20	2	31	55	72
	20	40	2	63	90	98
	40	60	1.5	55	85	95
	60	75	1.25	39	66	83
75	5	20	4	64	87	95
		15	3	42	65	79
	10	30	3	69	91	98
		20	2	30	49	64
	20	40	2	55	82	94
	40	60	1.5	44	73	89
	60	90	1.5	89	99	99

* RR is relative risk

Table 2. Proportional Hazards Model, 5 years, 3 centers

Prop. with event in 5 years	Prop. with risk factor	RR*	Power Percent N=125	N=250	N=375
.1	.1	3	22	40	55
		4	32	54	75
	.25	3	30	54	71
		4	42	70	99
	.5	3	30	53	70
		4	42	70	86
.2	.1	3	39	68	84
		4	57	87	96
	.25	2	26	47	63
		3	54	83	95
	.5	2	29	51	69
		3	55	84	95
.3	.1	2	25	45	61
		3	39	85	95
	.25	2	37	64	81
		3	73	95	99
	.5	2	42	69	86
		3	74	95	99
.4	.1	2	31	57	75
	.25	2	48	77	91
	.5	2	53	82	94
.5	.1	2	38	67	83
	.25	2	56	86	95
	.5	2	64	90	98

* RR is hazards ratio

4. COMPENSATION

   Ten dollars will be provided for each visit at which a swab for an anal cytology and anal HPV specimen is obtained. Depending on the site, twenty to thirty dollars will also be provided for each visit at which high resolution anoscopy is performed.

5. CONSENT

   Informed consent for the anal study will be obtained separately from the WIHS consent.

   WIHS has developed a counseling protocol in conjunction with the National Community Advisory Board for informing participants of the anal study (see Appendix A). This counseling includes information regarding what HPV is and what a positive anal cytology and/or biopsy means, as far as current understanding permits.

6. CLINICAL DATA COLLECTION

   a. Medical history of prior anal cancer or ASIL will be collected during the anal study interview. Additionally, in a supplemental questionnaire for the anal study, all participants will be asked in detail about their history of anal sexual activity and other potential risk factors for prevalent and incident anal HPV infection and/or lesions.

b. WIHS physical examination data will be used to identify peri-anal warts. Data from the WIHS history and physical examinations including cervical cytology, colposcopy and cervical HPV will be used in the analysis of the anal study results.

F. SPECIMEN COLLECTION

1. ANAL SWAB

a. An anal swab will be inserted into the anal canal and will then be inserted into the Thinprep™ cytology sample medium. After vigorous shaking, the swab should be removed and the cap on the vial tightly sealed.

b. Participants whose anal cytology is abnormal will be referred to a local clinician for high resolution anoscopy and biopsy of visible lesions. Biopsy tissues will be placed in a vial containing formalin. If the lesion is large enough, a second biopsy will be frozen in liquid nitrogen immediately and stored until shipment in a –70 degree freezer. The specimen will be shipped to San Francisco on dry ice.

c. These procedures will be repeated every six months. Women shown to have a high-grade lesion on cytology or biopsy will be withdrawn from the study and referred to their primary care provider and an anal surgeon for further follow-up and/or therapy.

d. Anal cytology specimens are placed at room temperature into the shipping box provided by the anal study coordinator in San Francisco. When the box is full the shipment will be sent. Anal biopsy specimens will be fixed in formalin and shipped to San Francisco. Frozen biopsies will be shipped on dry ice to San Francisco.

2. SHIPPING INFORMATION

a. Palefsky group sends to the sites Thinprep™ vials, packing boxes and pre-labeled FEDEX forms. The vials will be sent to:

Karlene Schowalter
2020 West Harrison St.
Room 2-208
Chicago, IL 60612
Phone: (312) 572-4546
Email: kschow@corecenter.org

Barbara Driscoll, RN
SUNY Downstate
450 Clarkson Avenue, Room BSB 3-109
Brooklyn, NY 11203
Phone: (718) 270-3310

Sharon Alpert
UCSF
AC-16
Phone: (415) 476-9356

b. Holly group sends interview and specimen tracking forms to sites with pre-labeled FEDEX forms. The forms will be sent to:

Karlene Schowalter
2020 West Harrison St.
Room 2-208
Chicago, IL 60612
Phone: (312) 572-4546
Email: kschow@corecenter.org

Barbara Driscoll, RN
SUNY Downstate
450 Clarkson Avenue, Room BSB 3-109
Brooklyn, NY 11203
Phone: (718) 270-3310

Sharon Alpert
UCSF
AC-16
Phone: (415) 476-9356

c. When Thinprep™ vials fill the vial tray (25 vials), the site ships the box with specimen tracking forms using the preprinted FEDEX labels to the following address:

Maria Da Costa
UCSF
521 Parnassus, Room C-233
San Francisco, CA 94143-0512
Phone: (415) 476-8885

Notify Maria of shipment by email: dacosta@cgl.ucsf.edu.

At UCSF, Thinprep™ vials and specimen tracking forms will be hand-delivered from the SF Specimen Bank to Maria Da Costa when the 25 vial tray is filled.

d. Interview forms from Chicago and Brooklyn (25 forms) are sent from the sites by

FEDEX to:

Jennifer Kristiansen
University of California San Francisco
3333 California Street, Suite 280
San Francisco, CA 94118-1944
Phone: (415) 476-3353

Interview forms from UCSF are picked up by Ivan Quesada (476-3345) every two weeks at 405 Irving Street.

e. Anal biopsies are sent in formalin or on dry ice with the specimen tracking forms by the sites to:

Maria Da Costa
UCSF
521 Parnassus, Room C-233
San Francisco, CA 94143-0512
Phone: (415) 476-8885

Notify Maria of shipment by email: dacosta@cgl.ucsf.edu.

f. Anal cytology and biopsy result forms will be faxed by Teresa Darragh to:

Claudia Ponath
UCSF
Fax: (415) 476-8528

Barbara Driscoll
SUNY Downstate
Fax: (718) 270-1685

Karlene Schowalter
Core Center, Chicago
Fax: (312) 572-4559

Email: kschow@corecenter.org

G. LABORATORY METHODS

1. SPECIMEN HANDLING

a. Anal cytology/HPV specimens:

After being transported to the local lab, Thinprep™ specimens can be stored at room temperature. When the box of vials is full, it can be shipped to San Francisco.

b. Anal biopsy specimens:

Anal biopsy specimens can be placed in a vial of formalin and shipped to San Francisco. Frozen biopsies should be immediately placed in liquid nitrogen, stored at –70 degrees until shipment and shipped to San Francisco on dry ice.

2. PCR ANALYSIS OF ANAL HPV DNA

To perform PCR on the Thinprep™ specimen, the specimen is vortexed for one minute. One ml of the solution is removed to a labeled microfuge tube with a transfer pipette. The eppendorf is spun at 14000 RPM for 15 minutes. The tubes are decanted and dried overnight or in a 65°C hot block for one hour. The pellets are resuspended in 100 µl Sample Transport Medium (Digene). Two µl of 10 mg/ml Proteinase K (Boehringer Mannheim, 200 µg/ml final concentration) are added. The samples are vortexed well and incubated at 56°C in a waterbath for one hour to digest the sample. The PK is inactivated and 5 ul of sample is used for PCR amplification using our standard protocol[16] and 40 cycles of PCR are performed. PCR products from positive samples will be typed by dot-blot hybridization using 39 individual type-specific probes.

3. HPV TYPE-VARIANT SEQUENCING

As part of the cervical HPV tests being performed in the WIHS, subjects positive for highly oncogenic types HPV 16, 18 and/or 31 will be tested to identify the HPV type-specific variants. We will use the same approach to test anal specimens. We will use direct sequencing to test the first of the series of specimens that was positive for the virus. Recent studies have suggested that non-European variants of HPV 16 and 18 are associated with increased risk of cervical and anal disease. [17, 18]

4. GENETIC ANALYSES OF ASIL TISSUES

We expect to obtain HSIL biopsies from at least 15 HIV- women and 150 HIV+ women, and will analyze as many as possible. We will use two general methods to analyze genetic changes in

ASIL and CSIL. These methods, Quantitative Microsatellite Analysis (QuMA) and Array-based Comparative Genome Analysis (ACGH), are described in the Preliminary Results. Briefly, QuMA is a technique in which PCR is monitored during the process of thermal cycling using an oligonucleotide homologous to microsatellite regions, e.g., $GT)_n$, and emits increasing fluorescence with each PCR cycle.[19] This process is an extension of the TaqMan analysis originated at Applied Biosystems [20] and it measures the copy number at specific microsatellite loci depending on the PCR primer pair used to amplify at each site. ACGH is a DNA microarray method in which a number of different bacterial artificial chromosomes (BACs) that have been mapped to specific regions of the human genome are isolated and micro-spotted onto microscope slides to allow comparative hybridization between two fluorescently labelled DNA samples, one from a test tissue and a second from normal tissue as a reference.[21] The relative intensities of the two fluorescent colors are measured for each BAC spot, yielding a measure of the relative amount of test to reference DNA in each region of the genome homologous to each BAC.

Although both techniques are designed to measure DNA copy number aberrations in test tissue, the amount of tissue DNA available for analysis determines which method to use. QuMA is usually performed using 15 ng of tissue DNA per regional locus, whereas ACGH requires about 1 microgram of tissue DNA regardless of the number of loci being analyzed. If fewer than 60 loci are being analyzed, QuMA is more frugal (60 X 0.015 = 0.9 micrograms).

Under these conditions, we will perform ACGH on DNA from the majority of biopsies, followed by more intensive comparison at specific loci between samples using QuMA for regions of common alteration. From our experience with ASIL in MSM, we expect this to include chromosome arm 3q, for which we already have 25 primer pairs.

H. DATA MANAGEMENT

All completed anal study data collection forms should be sent to the San Francisco site per the shipping information in Section F. In addition, the Recruitment Outcome and Disenrollment Forms should be locally entered into Apollo so that persistent tables will reflect accurate substudy enrollment information. All data will be imported into SAS for analysis.

I. REFERENCES

[1] Holmes F, Borek D, Owen-Kummer M, Hassanein R, Fishback J, Behbehani A, Baker A, Holmes G: Anal cancer in women. Gastroenterology 1988,95:107-111.

[2] Melbye M, Rabkin C, Frisch M, Biggar RJ: Changing patterns of anal cancer incidence in the United States, 1940-1989. American Journal of Epidemiology 1994, 139:772-80.

[3] Daling JR, Weiss NS, Hislop TG, Maden C, Coates RJ, Sherman KJ, Ashley RL, Beagrie M, Ryan JA, Corey L: Sexual practices, sexually transmitted diseases, and the incidence of anal cancer. N Engl J Med 1987, 317:973-7.

[4] Goedert JJ, Cote TR, Virgo P, Scoppa SM, Kingma DW, Gail MH, Jaffe ES, Biggar RJ: Spectrum of AIDS-associated malignant disorders. Lancet 1998, 351:1833-9.

[5] Frisch, M., Biggar, R. J., Goedert, J. J. Human papillomavirus-associated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome, J Natl Cancer Inst 2001; 92: 1500-1510.

[6] Palefsky JM, Holly EA, Ralston ML, Arthur SP, Jay N, Berry JM, DaCosta MM, Botts R, Darragh TM: Anal squamous intraepithelial lesions in HIV-positive and HIV-negative homosexual and bisexual men: prevalence and risk factors. J Acquir Immune Defic Syndr Hum Retrovirol 1998,17:320-6.

[7] Palefsky JM, Holly EA, Hogeboom CJ, Ralston ML, DaCosta MM, Botts R, Berry JM, Jay N, Darragh TM: Virologic, immunologic, and clinical parameters in the incidence and progression of anal squamous intraepithelial lesions in HIV-positive and HIV-negative homosexual men. J Acquir Immune Defic Syndr Hum Retrovirol 1998,17:314-9.

[8] Palefsky JM, Holly EA, Ralston ML, Jay N: Prevalence and risk factors for human papillomavirus infection of the anal canal in human immunodeficiency virus (HIV)-positive and HIV-negative homosexual men. J Infect Dis 1998,177:361-7.

[9] Palefsky JM, Holly EA, Ralston ML, Jay N, Berry JM, Darragh TM: High incidence of anal high-grade squamous intra-epithelial lesions among HIV-positive and HIV-negative homosexual and bisexual men. Aids 1998, 12:495-503.

[10] Heard I, Schmitz V, Costagliola D, Orth G, Kazatchkine MD: Early regression of cervical lesions in HIV-seropositive women receiving highly active antiretroviral therapy. Aids 1998, 12:1459-64.

[11] Ahdieh Lea. The effect of highly active antiretroviral therapy on cervical cytologic changes associated with oncogenic HPV among HIV-infected women. 18th International Papillomavirus Conference. Barcelona, Spain, July 23-28, 2000:Poster 073.

[12] Uberti-Foppa C, Lillo F, Ferari D. Longitudinal study on the effect of highlly active antiretroviral therapy on the clinical outcome of HPV-related lesions in HIV-positive women. Abstract TuPeB3167. XIII International AIDS Conference, July 9-14, 2000. Durban, South Africa, 2000:349.

[13] Koutsky L, Galloway DA, Holmes KK: Epidemiology of genital human papillomaviruses. Epidemiol Rev 1988, 10:122-163.

[14] Palefsky JM, Holly EA, Ralston ML, Da Costa MM: 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, 183:383-91.

[15] Holly EA, Ralston ML, Darragh TM, Greenblatt RM, Jay N, Palefsky JM: Prevalence and risk factors for anal squamous intraepithelial lesions in women. J Natl Cancer Inst 2001,; 93: 843-849.

[16] Palefsky JM, Minkoff H, Kalish LA, Levine A, Sacks HS, Garcia P, Young M, Melnick S, Miotti P, Burk R: Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high-risk HIV-negative women [see comments]. Journal of the National Cancer Institute 1999, 91:226-36.
[17] Xi LF, Koutsky LA, Galloway DA, Kuypers J, Hughes JP, Wheeler CM, Holmes KK, Kiviat NB: Genomic variation of human papillomavirus type 16 and risk for high grade cervical intraepithelial neoplasia [see comments]. Journal of the National Cancer Institute 1997, 89:796-802.
[18] Xi LF, Critchlow CW, Wheeler CM, Koutsky LA, Galloway DA, Kuypers J, Hughes JP, Hawes SE, Surawicz C, Goldbaum G, Holmes KK, Kiviat NB: Risk of anal carcinoma in situ in relation to human papillomavirus type 16 variants. Cancer Res 1998, 58:3839-44.
[19] Gibson UEM, Heid CA, Williams MP: A novel method for real time quantitative RT-PCR. Genome Research 1996, *6*: 995-1001.
[20] Breslow NE, Day NE: Statistical methods in cancer research. Vol 2- The design and analysis of cohort studies. International Agency for Research on Cancer Scientific Publications No. 82 1987.
[21] Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo W, Chen C, Zhai Y, Dairkee S, Ljung B, Gray J, Albertson D: High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nature Genetics 1998, *20*:207-11.

APPENDIX A: ANAL STUDYCOUNSELING PROTOCOL

Your Anal Study Results from the WIHS

What they mean to you

Words to know:
Human papillomavirus (HPV): A virus that is one of the most common sexually transmitted agents. Most women acquire HPV at some point during their lives, and in most women, the virus is harmless. In some women, however, the virus causes dysplasia or anal squamous intraepithelial lesions (ASIL), a change in the lining of the anus. ASIL is also harmless in most women, but in a small number, it may lead to invasive cancer of the anus if it is left untreated. Women who are HIV-positive are at increased risk of having HPV found in their anus, and for developing ASIL.

ASIL: Caused by HPV, the changes found in ASIL range from warts or low-grade ASIL to high-grade ASIL, which is more advanced. Warts or low-grade ASIL do not progress to cancer but a small number of women with high-grade ASIL may progress to cancer if it is not treated. Anal cytology (or Pap smears) is used as one of the tests to detect ASIL. It consists of a swab inserted into the anal canal to collect cells from the lining. The cells are then examined under a microscope to look for the changes of ASIL. Some women have an abnormality on their cytology called "atypia", also known as "atypical squamous cells of undetermined significance (ASCUS). Atypia is an in-between category- neither entirely normal nor severe enough to be called ASIL. If the cytology is abnormal, the next step in the investigation is to look directly for the area of the anal canal from which those abnormal cells came. To do this, a procedure called high resolution anoscopy (HRA) is performed. During HRA a scope is inserted into the anus and if the abnormal area is seen, a small piece of tissue (a biopsy) is obtained. The biopsy is then examined under the microscope to determine whether there is low-grade or high-grade dysplasia.

Little information is currently available about the course of anal HPV infection or ASIL in women. The purpose of this study is to better understand who is at risk for getting anal HPV infection and ASIL, and who is at risk for developing high-grade ASIL. The investigators also want to understand how changes in the cells of the anus caused by HPV may lead to development of ASIL. It is hoped that learning more about these changes will lead to new ways to predict who will progress to high-grade ASIL or cancer, and possibly new approaches to treating ASIL.

Summary:
- HPV is a very common virus in the cervix and anus. It causes no problems in most women. However, HIV-positive women are more likely to have both anal HPV infection and ASIL than HIV-negative women.
- Anal HPV infection may lead to development of ASIL in some women.
- Most women with ASIL will not develop anal cancer but a small proportion of women with high-grade ASIL may develop anal cancer if it is not treated.

ASIL is detected using a combination of anal cytology and high-resolution anoscopy
- Your medical provider or WIHS clinician can answer your questions or concerns about your test results.
- We will give you more information about anal HPV and ASIL as we learn about it – check your newsletters from your site.