

WOMEN'S INTERAGENCY HIV STUDY

SECTION 1: STUDY PURPOSE

The purpose of the WIHS is to comprehensively investigate the impact of HIV infection in women.

PRIMARY RESEARCH AREAS

- Spectrum and predictors of AIDS-defining and other HIV-related conditions.
- Predictors of cervical neoplasia, HPV-mediated cervical disease, and genital infections.
- Immunological and virologic markers and clinical predictors of disease progression.
- Trends in substance abuse, sexual behavior, health care utilization, and psychosocial outcomes.

A. WIHS-V AIMS AND HYPOTHESES

SOCIAL AND BEHAVIORAL SCIENCE SPECIFIC AIMS

Aim 1: Assess the contributions of substance use, mental health, violence, and other social, psychological, and behavioral factors to HIV-related quality of life (overall quality of life, general health, role function, pain, emotional well-being, energy/fatigue) and to women's ability to carry out social and self care-taking tasks.

Aim 2: Characterize the spectrum of women's engagement in HIV care (retention in care, ART treatment, ART medication adherence, viral load suppression) and explore social determinants, identify psychosocial and behavioral factors that account for variations in each component of the engagement spectrum, including characteristics of the built environment, organizational and provider factors, stigma, incarceration, housing instability, food insecurity, social support and loneliness, spirituality, transportation barriers and access to care, abuse history, depression and anxiety, and beliefs surrounding HIV care, empowerment, and activation.

Aim 3: Identify social, network, psychological, and behavioral factors associated with sexual health, engaging in sexual risk behaviors and, using HIV biomedical prevention strategies among women with and without HIV-infection and, assess how self-reported behavioral factors (sexual, drug and adherence practices) may be validated with more objective outcomes.

Aim 4: Assess the effectiveness of strategies designed to reduce the impact of HIV comorbidities, including smoking cessation, medication-assisted treatment for substance abuse, other treatment for substance abuse, mental health treatment, diet/exercise, housing support, and other health services interventions, and examine how multiple treatment regimens for comorbid conditions interact with HIV-related behavioral and clinical outcomes.

Aim 5: Engage in interdisciplinary collaborations to analyze the contribution of social and behavioral science theory and methods to the understanding of key neurocognitive, cardiovascular, metabolic, liver-associated, cancer, and other HIV-related outcomes across the lifespan.

EPIDEMIOLOGY SPECIFIC AIMS

Aim 1: Describe the treated history of HIV in U.S. women.

- 1a. Characterize changes in the use and effectiveness of HIV therapy over time.
- 1b. Characterize the effect of aging and the menopausal transition on the course of HIV disease and response to therapy.

1c. Characterize the influence of socioeconomic status, geographic and structural community factors and income inequality on HIV-associated outcomes.

1d. Develop innovative metrics to quantify the risk of morbidity and mortality among HIV-infected women, including established predictors and predictors differentially affecting women such as psychiatric co-morbidities, domestic coercion and gender-based violence.

1e. Characterize the attributable risk of race and ethnicity on HIV-associated outcomes using both self-categorized race and ancestry informative markers (GWAS, available March 2013).

1f. Characterize the attributable risk of markers of hemostasis (D-Dimer, PAI-1, Protein S and Factor VIII) on AIDS and non-AIDS deaths.

Aim 2: Define predictors of and barriers to optimal HIV therapy use among HIV-infected US women, including individual-level factors, community factors and the influence of local and national laws, policies and guidelines on access to and use of HIV therapy.

Aim 3: Define the clinical manifestations of established and emerging co-infections in the modern HAART era, and characterize their effect on the clinical course of HIV.

Aim 4: Develop novel epidemiologic and statistical methods for using observational cohort data to optimally estimate the effects of interacting exposures across time on clinical outcomes.

Aim 5: Refine methods of ascertaining and defining clinically relevant outcomes in an interval cohort.

Aim 6: Contribute data and methodologic expertise to national and international collaborative cohorts such as NA-ACCORD.

GYNECOLOGY-RELATED SPECIFIC AIMS

- 1) To determine the age of menopause for the cohort to allow coordination of studies assessing effect of menopause on HIV disease, pharmacology, coinfections, the vaginal ecosystem and complications.
- 2) To identify how gaps in contraception for WIHS participants have impacted HIV-infected women and their family planning choices, including pregnancy outcomes and permanent sterilization, and to evaluate the effect of effective contraception on consistent condom use.
- 3) To assess the effects of pregnancy and hormonal contraception on HIV disease progression and response to antiretroviral therapy.
- 4) To assess pathways to safe conception for HIV-infected women.
- 5) To assess risk factors for persistent/recurrent bacterial vaginosis, and other changes in the vaginal ecosystem.
- 6) To describe the prevalence, correlates, and consequences of reproductive coercion among HIV-infected and uninfected women
- 7) To describe the impact of childhood/adult sexual trauma and intimate partner violence on sexual and reproductive health among HIV-infected and uninfected women.
- 8) To assess the impact of genital tract trauma on the immune microenvironment of the female genital tract, including the effect of HIV status.
- 9) To assess hormonal regulation of intrinsic anti-HIV activity in genital secretions of postmenopausal women.
- 10) To assess the link between pregnancy outcomes (e.g., birth weight) and subsequent maternal health markers such as cIMT.

- 11) To assess the association and interaction between mental health (psychosocial stress, PTSD & depressive symptom burden), & substance abuse (crack/cocaine/heroin, marijuana, smoking, alcohol), with pregnancy outcomes (spontaneous abortion, preterm delivery, birth weight) and subsequent maternal health markers such cardiovascular/metabolic risk (carotid intimal medial thickness, fasting insulin/glucose), immunologic/virologic response to HAART, and quality of life/post-partum mental health/functional status.

HOST GENOMICS SPECIFIC AIMS

Aim 1. To test novel host genetic and/or genomic hypotheses addressing the four priority research areas of the WIHS:

Aim 1a) HIV Progression and Pathogenesis (including responses to treatment);

Aim 1b) Metabolic, Renal and Cardiovascular Disease;

Aim 1c) Neurocognition and Aging (including neurovascular diseases, mental illness, and dementias); and

Aim 1d) HPV Infection and Cancers.

Aim 2. To substantially improve and expand the WIHS research platform by accelerating the completion of host genetic and genomic studies addressing, but not limited to, the 4 priority research areas of the WIHS by:

Aim 1a) Identifying the subset of polymorphisms that are informative in the WIHS cohort thus guiding subsequent genome-wide association studies; and

Aim 1b) Building upon the Genome-Wide Association Study (GWAS) recently conducted for WIHS by the SFWIHS site to provide optimal access to genetics data and consistent analytic/informatics assistance to WIHS-associated and nonaffiliated investigators based on priorities governed by operating principles and the WIHS Executive Committee.

HPV & CANCER RESEARCH AIMS

I. HPV in Anogenital Neoplasia and the Oropharynx

I.a. Studies of Cervical HPV

- 1. To determine the immune deficits that beyond total CD4 T-cell count drive the relationship of HIV with HPV infection and incident cervical precancer.** Total CD4 is only one biomarker of immune function in HIV(+) patients. Our recent pilot studies show that CD8+ elevation (evidence of activation), high regulatory T-cell (T-regs) count, and low plasmacytoid dendritic cell (pDC) levels may be associated with HPV persistence. The current project uses flow cytometry to study CD4 and CD8 differentiation phenotypes, Tregs, pDCs and other immune cells to determine their influence on the full natural history of HPV, including the incidence of precancer.
- 2. To assess levels of local immune cells in paraffin-embedded CIN and relate them to CIN-1 progression to precancer,** including immunohistochemistry staining for CD4+ and CD8+ T-cells (by differentiation phenotypes), Tregs, NK cells, B cells, macrophages, pDCs, and granzyme B (an indicator of cytotoxic activity).
- 3. To study polymorphisms in selected (candidate) immune genes associated with HPV persistence and incident cervical precancer risk** (e.g., HLA genotype and killer immunoglobulin-like receptor (KIR) polymorphism). **We will also exploit the recently completed GWAS in WIHS to, in an agnostic fashion, study additional genetic associations with HPV persistence and precancer.**

4. **To study and compare the effects of HIV and immune status on the long term natural history of HPV by type.** Our prior studies in WIHS showed that HPV16, the HPV that causes half of cervical cancers, was the least associated with changes in host immune status (e.g., CD4 count). This relative independence from host immune status has been interpreted by our group and others as evidence that HPV16 may have an innate ability to avoid the effects of immune surveillance. Recently, we presented data showing that this phenotype (ie, independence from host immune status) is shared by each of the most common HPV in the general population (explaining their high prevalence), and are now initiating analysis of the HPV gene variation related to this phenotype.
5. **To investigate HPV “latency and reactivation”, its relation with immune status, and with risk of precancer.** WIHS data showed subsequent detection of HPV types not found at earlier clinical visits, even after prolonged periods of celibacy; results suggesting reactivation of previously acquired HPV following a period of low HPV replication. Further, these episodes of reactivation correlated with immune status. However, the frequency and duration of reactivation, and its relation with precancer risk is unknown and will now be studied.
6. **To assess outcomes following treatment of cervical precancer, including the rate of treatment failure and precancer recurrence after successful treatment, as well as the predictors of treatment failure and recurrence.** Variables will include the type of treatment used, lesion size, clear margins, immune status and, depending on additional funding, type-specific HPV persistence and other biomarkers (*similar to I.b.*, below).
7. **To assess the long term cumulative incidence of HPV-related precancer and cancer, and their risk factors.**
8. **To examine women’s knowledge of and attitudes toward HPV, HPV testing, and HPV vaccination.**

1.b. Molecular Methods for Cervical Cancer Screening

9. **To measure and compare the sensitivity/specificity/PPV/NPV of promising molecular assays for detection of CIN-2+/CIN-3+ as an adjunct to Pap tests in HIV(+) women.** At each clinical visit nearly a third of HIV(+) women have abnormal Pap tests, but most of these do not reflect clinically relevant disease. Thus, most colposcopy/biopsy is conducted unnecessarily. Encouragingly, a new era of molecular screening with evidence of greater sensitivity/specificity has begun. This study will assess (i) *several assays for detection of oncogenic HPV DNA, including a well established in-house PCR assay able to provide semiquantitative results for >40 individual HPV types;* (ii) *cellular markers of E6 activity / proliferation (p16/ki-67 cytology; CINtec+),* (iii) *cellular markers of aberrant S-phase (MCM2/Top2A cytology; BD ProExC),* (iv) *oncHPV E6/E7 oncogene mRNA expression (PreTect HPV-Proof).*
10. **To determine the optimal cervical cancer screening approach in HIV(+) women achievable using these several assays alone or in combination with one another.**
11. **To estimate cost-effectiveness of these approaches for cervical cancer screening in HIV(+) women.**

1.c. Extra-cervical Anogenital HPV and HPV-related Neoplasia

12. **To study the prevalence, long term cumulative incidence, response to treatment and risk of recurrence, of vaginal intraepithelial neoplasia (VAIN), vulvar intraepithelial neoplasia (VIN), and anogenital warts, and the relation of these events with host immune status as well as the use of HAART.**

13. To analyze prospective WIHS data regarding anal HPV and anal Pap/biopsy results, to assess the incidence of anal intraepithelial neoplasia (AIN), it's relation with HPV, and host immune status.

I.d. Oral HPV Natural History

14. To evaluate the effect of HIV and related immunosuppression on oral HPV persistence in the HAART era.
15. To evaluate whether HPV16 viral load is a predictor of persistent infection.
16. To explore behavioral (tobacco, marijuana, cocaine use and sexual behavior) and other (age, gender, race) characteristics which influence the persistence of oral HPV infection and examine whether HIV modifies these relationships. We will address the hypothesis that increasing age, tobacco and recreational drug use have an immune suppressive effect and may increase oral HPV persistence and re-expression.

II. Other Tumors and Malignancies

We will continue to identify and confirm all cancers that occur in WIHS participants using the following ascertainment methods: (1) searches of statewide cancer registries, (2) medical record confirmation of self-reported cancer diagnoses, and (3) WIHS-initiated gynecologic biopsies. Cancers that are initially identified by self-report or death certificate via a National Death Index (NDI) search will be classified as 'confirmed cancers' only if they are subsequently confirmed by medical record review or cancer registry matching. The state cancer registry matches are performed every two years and the NDI is matched every year. WIHS continues to be a major donor to the ACSR of tissue, blood, and oral rinses from HIV(+) and a comparative group of HIV(-) women. Our protocol involves donations from at least 10 women per WIHS site, per WIHS visit, who undergo colposcopy. The following is a list of the overarching aims of the Cancer Working Group,

1. To determine if the incidence of specific types of cancers is increased among HIV(+) as compared to HIV(-) women and the general population, and whether cancer incidence and survival are affected by use of HAART.
2. To investigate risk factors for specific cancers (breast, cervical, lung cancer, and non-Hodgkin lymphoma) with an emphasis on potential strategies for cancer prevention and cancer therapy.
3. To collect fresh-frozen tissue from biopsies of the cervix, vagina, and vulva and accompanying blood specimens and oral rinses for donation to the NCI-sponsored AIDS Cancer and Specimen Resource (ACSR). For selected malignancies, to collect and donate paraffin-embedded formalin-fixed tissue specimens from WIHS women with confirmed incident cancers.

Collaborations: Several new cancer collaborations have been established in recent years and will play a major role in WIHS V, including those with the HERS, MACS and NA-ACCORD. In addition, during WIHS V additional cancer collaborations with cohorts in Europe (HICDEP) and in Africa (IeDEA) are being developed.

LIVER DISEASE AND VIRAL HEPATITIS SPECIFIC AIMS

Aim 1. To define the hepatitis B and C status of all women in the WIHS cohort by the best available methodologies. To determine differences in prevalence and clinical manifestations of viral hepatitis by region, race/ethnicity and age.

Aim 2. To determine clinical and non invasive markers that best predict the rate of progression of liver disease in women in WIHS, including those who are co-infected with chronic HCV in order to estimate the fibrosis status of women over time.

While many ongoing and future WIHS analyses will make use of these precise liver predictor and outcome data, accomplishment of these core aims will support the specific scientific aims of WIHS V, including those related to the Pathogenesis, Cardiovascular/Metabolic, Genetics, Cancer and Neurocognitive Working Groups.

In addition, the following proposed liver-specific projects will be addressed by these core aims in WIHS V:

- To determine the predictive value of elastography and other non invasive serum measures of fibrosis to assess the trajectory of fibrosis progression.
- To determine the role of reproductive aging on liver disease progression using serum markers and transient elastography
- To characterize the interaction between HIV and viral hepatitis.
 - Determine the role of HIV in liver disease progression among co-infected women
 - Determine the effect of viral hepatitis on HIV disease status
- To determine the role of steatosis on liver fibrosis progression
- To determine the role of HCV on cardiovascular disease
- To determine the role of HCV on neurocognitive function
- To determine the genetic predictors of clearance of HCV
- To determine the genetic predictors of HCV disease progression

CARDIOVASCULAR AND METABOLIC AIMS

Aim 1. To build upon the existing WIHS metabolic and vascular studies to assess the contributions of factors that likely influence vascular, bone, and kidney injury including: somatic and gonadal aging (as measured by AMH levels), host genetic factors, behavioral factors (recreational drug use, smoking), metabolic factors (adiposity, sarcopenia, insulin sensitivity, diabetes, and dyslipidemia), and HIV-related factors (immunosuppression, inflammatory mediators, coinfections, and exposure to specific ARVs). Hypotheses include:

- a) HIV infection will be independently associated with increased symptomatic and asymptomatic peripheral arterial disease (PAD) measured using the ankle brachial index (ABI). The association will be only partially explained by smoking and metabolic factors (specifically adiposity, diabetes, insulin resistance, and dyslipidemia) associated with HIV infection.
- b) Among HIV-infected women, the severity of immunosuppression and elevations in markers of inflammation will be associated with lower ABI values and increased symptomatic and asymptomatic PAD after controlling for demographic, behavioral, and metabolic factors.
- c) Greater carotid intima thickness (CIMT), a marker of atherosclerosis will be associated with abnormal ABI values.
- d) Longitudinal exposure to metabolic and vascular risk factors (that may be elevated in HIV) and viral related risk factors will be associated with specific patterns of kidney injury.
- e) During the menopausal transition, HIV-infected women will have higher rates of bone loss than HIV-uninfected women, and also than premenopausal HIV-infected women. The association will be explained by increased bone turnover, inflammation, and decreased lower extremity muscle

mass in the setting of HIV infection and menopause.

- f) After the menopausal transition, HIV-infected women will have an increased risk of adiposity, diabetes, dyslipidemia, vascular injury and incident fractures that will be influenced by host genetic factors.

Aim 2. To determine the association of HIV, vascular, bone, and kidney injury and their associated factors with functional declines (e.g. kidney, neurocognition and physical function). Hypotheses include:

- g) HIV infection will be associated with decreased physical function due to the catabolic effects of HIV on muscle mass in the lower extremities.
- h) Lower ABI values will be associated with decreased physical function due to decreased lower extremity muscle mass.
- i) Lower ABI values may also be associated with decreased kidney and neurocognitive function due to decreased perfusion.
- j) Kidney injury markers resulting from metabolic, vascular and HIV-related factors will be strongly and independently associated with declining kidney function
- k) HIV-associated bone loss and sarcopenia will be associated with greater declines in physical function.

NEUROCOGNITION SPECIFIC AIMES

1. To implement a cost-effective, validated process for the evaluation and identification of HAND into the WIHS and to determine rates of HAND in HIV-infected women.

Hypothesis 1: About 47% of HIV-infected women in the WIHS will meet algorithmically-determined criteria for HAND, including HIV-1 Associated Asymptomatic Neurocognitive Impairment, Mild Neurocognitive Disorder, and Dementia.

2. To provide original insights into the prevalence and predictors of HAND and cognitive function in the largest cohort of HIV+ women studied to date. Predictors are obtained through CORE activities and focus on reproductive aging, menopausal symptoms, mood disorders, early-life trauma, specific genetic factors and related biomarkers as key sex-relevant determinants of HAND in HIV+ women.

Hypothesis 2a: A CIDI-based diagnosis of mood disorder (e.g., post-traumatic stress disorder, major depression), elevated psychosocial stress, elevated depressive symptoms and early life trauma will relate to worse performance and a greater rate of cognitive decline on prefrontal-mediated tasks and HAND. The magnitude of the effect on verbal memory will be greater for HIV+ versus HIV- women.

Hypothesis 2b: After adjusting for age, deficits in delayed verbal memory will increase as women age reproductively and transition through the menopause. These deficits will be greater in HIV+ versus HIV- women. This pattern of effects will be evident when new state-of-the-science bleeding and endocrine (e.g., antimullerian hormone) criteria are used to accurately stage reproductive aging in HIV+ women.

Hypothesis 2c: Menopause-related mood symptoms, particularly anxiety, will be associated with deficits in verbal memory as women transition through the menopause. This relationship will be stronger in HIV+ versus HIV- women.

Hypothesis 2d: There are regions in the genome that will be associated with an increased risk of HAND. Among the candidate genes linked to HAND will be polymorphisms in genes linked to: a) the production of chemokines, chemokine receptors, and other cytokines (e.g., chemokine receptor 5, stromal cell-derived factor-2); b) neurophysiology, neuromodulation, and

neurotransmission (e.g., Catechol-O-Methyltransferase, apolipoprotein); c) Sex-relevant genetic polymorphisms including genetic polymorphisms in estrogen receptor alpha and beta and genetic polymorphisms associated with sex-specific risk of PTSD; and d) genes related to metabolism, adipokines, and other risk factors for obesity and metabolic syndrome

Hypothesis 2e: Substance use, particularly use of cocaine, will predict longitudinal changes in verbal memory and executive function, and this effect will be greater in HIV+ versus HIV- women.

Hypothesis 2f: Cardiovascular risk factors and insulin resistance will predict cognitive impairment in HIV-infected women.

Hypothesis 2g: Metabolic risk factors (visceral adiposity, BMI) will predict cognitive dysfunction in HIV-infected women.

Hypothesis 2h: Cognitive impairment and HAND will relate to women's ability to carry out social, self-care and family life tasks, such as health care activities, employment, and family roles. (Weber & Schwartz)

PATHOGENESIS SPECIFIC AIMS

Aim 1: To further analyze soluble and cellular immunology biomarkers that have been obtained on women in the WIHS that represent key HIV disease phenotypes and determine which immunologic changes predict HIV and non HIV related co morbidities independent of existing clinical disease markers of HIV disease progression (CD4+ cell count and plasma RNA).

We will analyze biomarker data obtained during WIHS IV that include soluble biomarkers (pro and anti inflammatory cytokine, chemokine, and growth and metabolic factor) cellular phenotypes that include T cell maturation subsets, regulatory T cell populations, immune activation and apoptosis markers, and functional T cell subpopulation (TH1, TH2, TH17) and markers of microbial translocation (Sol CD14, LBP, EndoCab, and IFABP). We will evaluate comparison groups defined by HIV progression and treatment response including “elite” controllers, long term non progressors, untreated progressors, HAART treated viral and immune responders and non responders and HIV uninfected women.

Aim 2: We propose to evaluate immunologic, virologic and pharmacologic parameters in the female genital tract of women in the WIHS. This aim will need development following discussion with the WIHS EC.

PHARMACOLOGY WORKING GROUP AIMS

Aim 1: To continue ongoing WIHS work in estimating long-term exposure to ARVs via hair levels and to analyze hair concentrations of ARVs as contributors to key HIV-related outcomes

Hypothesis: Hair concentrations of ARVs in the WIHS, demonstrated to be strong independent predictors of treatment outcomes to date, will continue to serve as important biomarkers of adherence and exposure to ARVs in multiple WIHS analyses

Approach: We will continue to measure concentrations of the anchor PI, NNRTI and/or integrase inhibitor for each HIV-positive woman on treatment during WIHS V. We propose simultaneous assessment of time-varying cumulative measures of ARV exposure (using area under the curve of hair concentrations) and cumulative measures of plasma HIV exposure (using area under the viral load curve) in multivariate models examining key HIV outcomes available in the WIHS to elucidate the contributions of ARV exposure versus HIV replication itself on long-term complications/outcomes of chronic infection, including neurocognition measures, renal function, hepatic fibrosis, bone mineral density, metabolic outcomes, and indicators of vascular injury. We will interface with the following WIHS working groups to use hair ARV levels a predictor of relevant outcomes in WIHS: Pathogenesis, Neurocognition, Metabolic (including Renal and Cardiovascular), and Cancer. We will

also work with the Behavior Working Group to include hair measures of ARVs as markers of adherence as an outcome in various analyses examining sociodemographic and behavioral predictors of adherence.

Aim 2: To institute hair collection for participants on tenofovir (TFV)-based regimens and to assess TFV exposure via hair measures as a contributor to renal outcomes in the WIHS

Hypothesis: Given the known impact of TFV on renal injury and proximal tubular dysfunction, a robust biomarker of TFV exposure using hair levels will improve predictive models of renal outcomes in the WIHS

Approach: For participants on TFV-containing regimens in WIHS V, we propose to collect 100 strands of hair for the measurement of TFV exposure once yearly (not at every visit). The approach to data analysis is thereafter similar to what was proposed for Aim 1, but renal outcomes, such as estimated glomerular filtration rates using creatinine and cystatin-C, as well as urinary biomarkers proposed by Dr. Michael Shlipak's group at UCSF, will be examined.

Aim 3: To develop hair assays for new hepatitis C medications coming into prevalent use in the WIHS

Hypothesis: Given the importance of novel oral hepatitis C (HCV) drugs entering the market for use in HIV-HCV co-infected patients and significant drug-drug interactions between these agents and ARVs, the development of hair assays for prevalent-use HCV agents during WIHS V will enable subsequent investigations to help explain treatment response and side effects, as has been done for ARVs.

Approach: To develop methods for the measurement of HCV protease inhibitors and other novel HCV treatments coming into prevalent use in the cohort, we will recruit HCV-monoinfected volunteers as donors of full head hair as follows: the full head hair donors must be receiving and responding to (verified by provider) boceprevir, telaprevir, a HCV NS5B polymerase inhibitor or other novel hepatitis C drug (currently in clinical trials). Full shaved hair specimens will be used for the development of new measurement assays of the novel HCV medication, including experimentation with extraction conditions and reagents.

Aim 4: To continue work modeling non-genetic and genetic contributors to ARV exposure estimated by hair concentrations and intensive pharmacokinetics (PK) data in the WIHS

Hypothesis: Multivariate modeling of non-genetic and genetic contributors to ARV exposure estimated by hair levels and intensive pharmacokinetics (PK) data in the WIHS will provide important information on factors contributing to inter-individual variability in exposure in real-world settings

Approach: We will continue our work to assess non-genetic and genetic factors that contribute to ARV exposure using the prior intensive pharmacokinetics data from WIHS IV and hair data from WIHS IV and V. After determining traits that influence areas-under-the-curve from intensive PK curves, we will assess impact on levels of the ARVs in hair, incorporating genetics data from the Genomics Wide Association Studies (GWAS) in WIHS.

Aim 5: To compare antiretroviral therapy (ART) concentrations in hair, plasma, cervical and vaginal tissue, as well as cervicovaginal fluid, in postmenopausal women in WIHS to assess rates of HIV viral persistence in the female genital tract

Hypothesis: Postmenopausal women will have a higher rate of HIV persistence despite higher ART exposure. ART alone is not able to overcome the pro-inflammatory environment associated with menopause.

Approach: UNC WIHS, Bronx WIHS and the UCSF WIHS sites will share specimens and data to address the comprehensive evaluation of genital tract ART exposure, mucosal immunity, and

virology that occur in response to reproductive aging. For this pharmacology aim, we will conduct a cross-sectional study in 40 HIV-infected women (20 premenopausal and 20 postmenopausal) who have been on ART for > 2 years who rapidly responded to ART (HIV RNA < 200 copies/ml after 6 months ART) and who have a viral load (VL) that is stably < 50 copies/mL. ART concentrations will be measured in six biological matrices—hair, plasma, PBMCs, vaginal tissue, cervical tissue and cervicovaginal fluid.

Virologic and immunological parameters will be measured at the Bronx WIHS site through the Bronx-UNC pathogenesis collaboration. We will then conduct multi-level analyses to evaluate viral persistence in the blood and genital tract (as measured by local and compartmental ARV exposure (pharmacokinetics), and immune activation and inflammation.) Using fluid and tissue concentrations of ARVs in addition to virologic and immunologic measures, we will develop a pharmacokinetics/ pharmacodynamics (PK/PD) model to determine a minimal ARV concentration (in each fluid/tissue) that fully suppresses replication in the female genital tract (FGT).

B. WIHS-IV CORE AIMS AND HYPOTHESES

PROJECT IA: HIV PATHOGENESIS

AIM 1. To identify key HIV disease phenotypes and assess the extent and patterns of immunologic perturbation and to determine whether these immunologic characteristics predict HIV-related outcomes independently of existing clinical disease markers of HIV disease progression (CD4+ cell counts and plasma HIV RNA concentration).

- **Hypothesis 1a.** Soluble and cellular immune responses are closely associated with clinical and virologic outcomes.
- **Hypothesis 1b.** Changes in soluble and cellular markers precede and predict transitions in HIV disease progression.

AIM 2. To determine whether reproductive aging (transition through perimenopause to menopause) modifies the association of SBM and cellular markers with HIV disease progression.

- **Hypothesis 2.** Reproductive Aging.

AIM 3. To evaluate the relationship between immunologic functions, HIV outcomes, antiretroviral drug exposure and reproductive aging on conditions associated with morbidity in HIV-infected women, including cardiovascular disease and metabolic abnormalities (Project II) and neurocognitive function (Project III).

AIM 4. To assess and compare HIV-associated changes in gut-associated lymphoid (GALT) and endometrial tissues.

- **Hypothesis 4.** Endometrial tissue can be used to evaluate mucosal lymphoid tissues in HIV pathogenesis research. HIV infection will be associated with reduced numbers of CD4 memory cells, increased lymphocyte activation, apoptosis, cellular turnover, T regs, and collagen deposition, in both gut and endometrium. The extent of the abnormalities will be directly correlated with intracellular levels of HIV RNA.

PROJECT IB: WIHS ANTIRETROVIRAL EXPOSURE PROJECTS

AIM 1. For WIHS-IV, we propose to perform intensive PK studies for two additional ARVs under conditions of actual use to identify factors that significantly influence exposure, including hepatic and renal function, reproductive aging, concurrent medications, dietary factors, illicit drugs, and host genotype.

- **Hypothesis 1a.** Relatively minor perturbations in hepatic and renal function, which are common in HIV-infected women, may result in significantly altered ARV exposure.
- **Hypothesis 1b.** Menopause and aging have a significant impact on ARV metabolism and women will have different levels of ARV exposure over the reproductive cycle and lifespan.
- **Hypothesis 1c.** Other factors will significantly contribute to variance in ARV exposure, which may include age, use of specific concurrent medications, dietary factors, ideal body weight, race and use of illicit substances.
- **Hypothesis 1d.** Genotypic variability in transporter and metabolic enzymes may have a large contribution to ARV drug exposure⁵⁶⁶, so the incorporation of pharmacogenomic parameters into intensive PK models will explain a greater proportion of inter-individual variability in exposure.

AIM 2. For WIHS-IV, we will incorporate longitudinal exposure measurements based on sparse drug levels with population PK modeling into predictive models of virologic and immunologic outcomes, as well as adverse effects, for participants on HAART.

- **Hypothesis 2a.** Population PK model-based estimates of HAART exposure will be a better predictor of HAART outcomes (including self-reported adverse effects) than self-reported adherence or single plasma ARV levels.
- **Hypothesis 2b.** Greater HAART exposure, as measured by population PK model estimates, will be predictive of better virologic control, a lower incidence of viral rebound and resistance, improved CD4 cell count recovery rates, and higher rates of lipid perturbations.
- **Hypothesis 2c.** Another method of assessing drug exposure (developed in the WIHS) is the measurement of ARV levels in hair, which have been found to be strong independent predictors of virologic success. Population PK model-based estimates of exposure will correlate well with hair measurements of ARVs.
- **Hypothesis 2d.** Women with good HAART outcomes despite low estimates of ARV exposure will demonstrate host immune response characteristics similar to natural controllers (Project IA).

PROJECT II: CARDIOVASCULAR AND METABOLIC DISORDERS

AIM 1. To examine the role of *chronic inflammation* and *immune function* in the development of fat and metabolic changes and CVD among HIV-infected and HIV-uninfected women.

- **Hypothesis 1a.** HIV will be associated with elevated inflammatory markers (e.g., serum CRP, IL-6, TNF- α) when compared to HIV-uninfected women; among HIV-infected women, elevations in inflammatory markers will be associated with risk and severity of peripheral fat loss, disorders in glucose and lipid metabolism, and subclinical atherosclerosis.
- **Hypothesis 1b.** Progression of HIV-associated peripheral fat loss (as measured by hip circumference and in a subset of women, by direct measures of leg fat), and increased obesity, particularly visceral obesity (as measured by the surrogate of waist circumference and in a subset of women, by direct measures of visceral adipose tissue volume) will be associated with changes in serum adipokines (e.g., decreases in adiponectin and leptin); these adipokine alterations may be associated with risk and severity of disorders in glucose metabolism, and subclinical atherosclerosis in HIV-infected women.

- **Hypothesis 1c.** Polymorphisms in genes involved in inflammatory cytokine activity, adipose tissue differentiation, and chemokine receptors will be associated with body fat changes, disorders in glucose and lipid metabolism, and subclinical atherosclerosis in HIV-infected women.
- **Hypothesis 1d.** Menopausal transition as measured by mullerian inhibiting substance (MIS), a biochemical marker of ovarian failure, and changes in sex hormone levels (lower levels of sex hormone binding globulin (SHBG), increases in testosterone, and decreases in dehydroepiandrosterone sulfate (DHEA-s)) will lead to further increases in CRP levels and risk of disorders in glucose and lipid metabolism, and subclinical atherosclerosis in HIV-infected women, even after adjustment for obesity and visceral obesity.
- **Hypothesis 1e.** Concurrent HCV infection will be independently associated with decreased CRP levels and more favorable lipid profiles, but increased insulin resistance and diabetes when compared to HIV-monoinfected women.
- **Hypothesis 1f.** HIV-infected women will demonstrate more rapid progression to osteopenia and osteoporosis (as measured by dual energy X-ray absorptiometry (DXA) scans in a subset of women) than HIV-uninfected women; among HIV-infected women, elevations in HIV-associated inflammatory markers, more rapid progression of HIV-associated fat loss, and changes in sex hormone levels with aging will be associated with worsening bone mineral density.

AIM 2. To assess the association of HIV infection and ARV use with endothelial dysfunction: brachial artery ultrasound (BAUS) measurements of flow-mediated vasodilation, circulating endothelial cells (CECs), and endothelial progenitor cells (EPCs).

- **Hypothesis 2a.** HIV infection, low CD4 count, ARVs, and traditional CVD risk factors all contribute to impaired endothelial function, an intermediate step in the development of atherosclerosis.
- **Hypothesis 2b.** Observed associations of endothelial dysfunction with low CD4 count, obesity, sex hormone levels, and other exposures are mediated by systemic inflammation, cytokines, and adipokines.
- **Hypothesis 2c.** Impaired endothelial function is associated with other co-morbidities including HCV coinfection, alcohol and drug use.
- **Hypothesis 2d.** The numbers of CECs/EPCs in peripheral blood will serve as a marker for endothelial damage and will correlate with endothelial dysfunction studies as measured by the gold-standard BAUS measurement.
- **Hypothesis 2e.** Markers of endothelial function are related to other subclinical CVD measures, including carotid intimal medial thickness (cIMT), arterial stiffness, and cardiac dysfunction.

AIM 3. To study the prevalence and predictors of cardiac structural and functional abnormalities in HIV-infected women.

- **Hypothesis 3a.** HIV infection is associated with left ventricular (LV) systolic dysfunction and increased LV mass.
- **Hypothesis 3b.** Among HIV-infected women, severity of HIV infection and concurrent HCV infection are associated with LV systolic and diastolic dysfunction.
- **Hypothesis 3c.** LV dysfunction is accompanied by increases in brain natriuretic peptide (BNP) levels.

PROJECT III: NEUROCOGNITIVE DISORDERS

AIM 1. To conduct a comprehensive longitudinal evaluation of neurocognitive function in HIV-positive women. (*Led by J. Manly and E. Martin*)

Aim 1.1. To determine if HIV-positive women experience more rapid age-related decline in a range of cognitive abilities as compared to risk-control-matched, HIV-negative women.

- **Hypothesis 1.1.1.** HIV-positive women will experience a more rapid age-related decline in speed, executive function, and memory.

Aim 1.2. To determine how the rate of cognitive change is influenced by the unique and synergistic effects of sociodemographics, substance misuse, laboratory indices of HIV disease severity, additional medical and psychiatric co-morbidities, and to determine whether these associations differ from those seen among HIV-positive women.

- **Hypothesis 1.2.1.** Fewer years of education; poorer quality of education, as estimated by reading achievement; lower estimated IQ; minority race/ethnicity; and increased age will predict faster rates of decline in working memory and verbal learning and memory over time.
- **Hypothesis 1.2.2.** Depressive symptoms, substance abuse, immunologic function, health, and exposure to antiretroviral therapy, analyzed as time-dependent covariates, will be independently associated with more rapid decline in executive function, learning efficiency, and delayed memory.

Aim 1.3. To investigate the normal range of scores on neuropsychological tests representing various cognitive domains among HIV-uninfected women and to determine: (1) cutoffs that define neuropsychological dysfunction after adjusting for the role of age and educational experience on test performance, and (2) definitions of asymptomatic neurocognitive impairment (ANI), HIV-associated mild neurocognitive disorder (MND), and HIV-associated dementia (HAD).

AIM 2. To characterize the impact of negative life events and self-reported post-traumatic stress disorder (PTSD) symptoms on increased vulnerability for neuropsychological dysfunction in HIV-infected and at-risk HIV-uninfected women. (*Led by C. Smith, K. Weber and P. Maki*)

Aim 2.1. To characterize the relationship between PTSD symptoms and neuropsychological test performance in HIV-positive and at-risk seronegative women.

- **Hypothesis 2.1.1.** The presence of clinically significant symptoms of PTSD will predict worse episodic memory, and the magnitude of this effect will be greater for HIV-infected versus HIV-uninfected women.
- **Hypothesis 2.1.2.** A history of negative life events, which includes childhood and/or sexual, physical, and emotional abuse, will predict worse episodic memory, and the magnitude of this effect will be greater for HIV-infected versus HIV-uninfected women.
- **Hypothesis 2.1.3.** Having experienced a greater number of negative life events will predict poor performance on measures of executive functioning, and the magnitude of this effect will be greater for HIV-positive versus HIV-negative women.

Aim 2.2. To examine the contribution of childhood trauma on the development and persistence of clinically significant symptoms of PTSD and depression in HIV-infected and HIV-uninfected women.

- **Hypothesis 2.2.1.** HIV-infected women with a history of childhood trauma are at increased risk for clinically significant symptoms of depression and PTSD.

AIM 3. To better understand the impact of menopausal stage, menopausal symptoms, and ovarian steroids on psychological health, cognition, and functional status in HIV-infected and HIV-uninfected women. (Led by P. Maki)

Aim 3.1. To examine the frequency and severity of menopausal symptoms in HIV-infected and HIV-uninfected women as a function of menopausal stage.

- **Hypothesis 3.1.1.** Vasomotor symptoms, psychological symptoms, insomnia, and genitourinary symptoms will be more common among midlife HIV-positive women compared with midlife HIV-negative women, particularly as women transition from the premenopausal stage to the perimenopausal stage.

Aim 3.2. To determine the extent to which the rate of decline in verbal memory and executive function is influenced by menopausal stage and menopausal symptoms.

- **Hypothesis 3.2.1.** Verbal memory and verbal fluency will worsen as women transition from the pre- to perimenopausal stage, even after controlling for age, disease progression, and other confounding factors.

Aim 3.3. To examine depressive symptoms in HIV-infected and HIV-uninfected women in relation to menopausal stage, and to relate this change in mood to change in cognition.

- **Hypothesis 3.3.1.** Both groups will show a significant increase in depressive symptoms as they transition from pre- to perimenopause.
- **Hypothesis 3.3.2.** The worsening of depressive symptoms during perimenopause will be related to a decrease in verbal memory and increase in executive dysfunction.
- **Hypothesis 3.3.3.** Severe perimenopausal depressive symptoms will relate to harmful behaviors (e.g., substance use and worse self-reported parenting skills).

AIM 4. To characterize sociodemographic, behavioral, and cognitive predictors of functional outcomes that are particularly relevant for HIV-infected women. (Led by K. Weber and R. Schwartz)

- **Hypothesis 4.1.** Overall and domain specific neurocognitive impairment and rate of cognitive decline will predict onset and severity of gender- and HIV-related functional disabilities, and this relationship will be moderated by household structure or functional and community factors.
- **Hypothesis 4.2.** Lower socioeconomic status, substance use, criminal involvement, symptoms of PTSD and depression, higher household burden, and cognitive impairment will predict lower self-ratings of parenting/grandparenting self efficacy and formal loss of child custody in HIV-infected and HIV-uninfected women.
- **Hypothesis 4.3.** Higher cognitive performance will predict return to work and maintenance of employment among HIV-uninfected women; however, HIV-infected women will report disincentives for employment including retention of disability income and childcare arrangements that would be barriers to employment even with good cognitive function.
- **Hypothesis 4.4.** After controlling for age and substance misuse, compared with HIV-negative women, HIV-positive women will score higher in Quality of Life (QOL) domains for social and role function, have less difficulty performing Instrumental Activities of Daily Living (IADL), report less criminal involvement, report fewer depressive and PTSD symptoms, and report higher self ratings of parenting skills and better maintain child custody; these differences will relate to greater health care utilization and access to support services among HIV-infected women.

- **Hypothesis 4.5.** Onset of functional disabilities will predict a more rapid subsequent trajectory of cognitive decline and will relate to decreased time spent outside the home, physical inactivity, and lower IADL scores; this relationship will be moderated by the presence or absence of supportive care provided by household members.

AIM 5. To integrate neurocognition and neuropathy data with data from other WIHS working groups to understand the etiology of cognitive decline and neuropathy in HIV-infected women. (Including past and future contributions from H. Crystal, R. Greenblatt, M. Young, V. Valcour, C. Smith, and other WIHS investigators)

Aim 5.1. To understand the etiology of cognitive decline in WIHS women.

Aim 5.2. To understand the prevalence and etiology of neuropathy. (Led by H. Crystal)

Aim 5.2.1. To understand the prevalence and incidence of neuropathy in WIHS women based on formal objective assessments.

- **Hypothesis 5.2.1.1.** The prevalence and incidence of neuropathy will be increased in relation to HIV and HCV infection status, and age.

Aim 5.2.2. To understand the influence of menopausal stage, inflammation, and cardiovascular disease on the prevalence and incidence of HIV-related distal sensory polyneuropathy (DSPN).

- **Hypothesis 5.2.2.1.** Because estrogen reduces neuroinflammation, DSPN will be less frequent (after adjusting for co-morbidities and age) in premenopausal women compared with postmenopausal women.
- **Hypothesis 5.2.2.2.** Although cardiovascular risk factors and inflammation have been associated with cognitive impairment, mechanisms explaining these associations are not fully understood. We hypothesize that risk factors for dysfunction in the central nervous system may also affect the peripheral nervous system and manifest as DSPN.

Aim 5.2.3. To examine the relationship between cognition and DSPN.

- **Hypothesis 5.2.3.1.** If common mechanisms lead to dysfunction in the central and peripheral nervous systems, then there should be a significant relationship between DSPN and cognition.

PROJECT IVA: HPV AND CERVICAL NEOPLASIA RESEARCH

AIM 1. To study and compare the effects of immune status (combined plasma HIV RNA/CD4+ strata) on the long-term natural history of HPV by type and phylogenetic group.

- **Hypothesis 1a.** There is broad variation in the effects of immune status on HPV infection by type, reflecting differences in the innate capacity of HPVs to avoid immune surveillance.
- **Hypothesis 1b.** These type-specific differences are genetically driven and will correlate, in part, with HPV phylogenetic group.

AIM 2. To assess the association of local cervical HIV RNA levels with risk of HPV infection.

- **Hypothesis 2.** The local immunologic milieu as reflected by cervical HIV RNA level is an independent predictor of HPV infection.

AIM 3. To determine the factors associated with development of severe cervical dysplasia among HIV-infected women.

- **Hypothesis 3.** Host immune status is a significant risk factor for development of severe cervical dysplasia. While the impact of immunodeficiency may be greater on the reactivation

of oncogenic HPV infection than on its persistence and progression, we anticipate that each of these factors will contribute to the overall risk of severe cervical dysplasia.

AIM 4. To study HPV vaccination in HIV-positive women, by monitoring the use of HPV vaccine in the WIHS cohort and assessing the safety, tolerability and immunogenicity of Gardasil HPV vaccine (Merck & Co.) in HIV-positive women (N=396 – a third to be enrolled through the WIHS) as part of a collaborative pilot study led by ACTG and in collaboration with the AIDS Malignancy Consortium (AMC).

- **Hypothesis 4a.** We hypothesize that few HIV-positive women who are not part of the clinical trial will receive HPV vaccine (pending the results of the vaccine studies), but if the sample size is large enough, we will assess whether those who are vaccinated have lower rates of infection with vaccine-related HPV types than those not vaccinated.
- **Hypothesis 4b.** We further hypothesize that Gardasil will be safe and well tolerated in HIV-positive women, as are many other vaccines, and will have immunogenicity in immune competent and some immunocompromised HIV-positive women.

PROJECT IVB: OTHER TUMORS AND MALIGNANCIES

AIM 1. To determine if there is an increased incidence of specific types of cancers among HIV-infected women as compared to HIV-uninfected women and to the general population, and whether cancer incidence and survival are affected by use of HAART.

- **Hypothesis 1a.** The incidence of AIDS-related cancers in both HAART users and non-users will be higher than expected among HIV-infected women, when compared to age- and race-adjusted HIV-uninfected WIHS women and to the sex-, age- and race-adjusted general population.
- **Hypothesis 1b.** The incidence of certain non-AIDS-related cancers (such as lung cancer) will be higher than expected among HIV-infected and HIV-uninfected WIHS women, when compared to age- and race-adjusted rates in the general population in both the early and current HAART eras.
- **Hypothesis 1c.** The use of HAART, and response to HAART, will be associated with a decreased incidence of KS and NHL but not HPV-related cancers such as invasive squamous cell cervical, anal, and vulvar cancer.
- **Hypothesis 1d.** Among women who develop cancer, HAART use will be associated with longer survival time following the cancer diagnosis.

AIM 2. To investigate risk factors for breast and lung cancer among HIV-infected women.

- **Hypothesis 2a.** The low incidence of breast cancer observed prior to 1998 in the WIHS has now approached the incidence rate observed in the general population. We hypothesize that the reason for this is widespread use of HAART leading to weight gain, rising levels of sex hormones and immune reconstitution.
- **Hypothesis 2b.** The elevated incidence of lung cancer found during prior years in the WIHS is associated with cigarette smoking and not immunosuppression. However, lung cancers in HIV-infected women occur at an earlier age and characteristics of disease differ from lung cancers in HIV-uninfected women. When compared to the men in the MACS, and after adjustment for smoking history, women have a higher incidence of lung cancer.
- **Hypothesis 2c.** The reduced risk of breast cancer and the elevated risk of lung cancer previously seen in the WIHS were due to a comparatively low risk profile of the WIHS participants for breast cancer and a comparatively high risk profile for lung cancer when compared to the general population.

AIM 3. To collect fresh-frozen tissue from biopsies of the cervix, vagina, and vulva and accompanying blood specimens and oral rinses for donation to the NCI-sponsored AIDS Cancer and Specimen Resource (ACSR).

CORE A: COLLABORATIVE CORE

AIM 1: To initiate and sustain successful scientific partnerships that utilize the full potential of WIHS data, specimens, and resources, thus leveraging the investing in WIHS to optimize the overall scientific gain in understanding HIV pathogenesis, clinical disease and treatment.

AIM 2: To establish and develop methods that will foster scientific communication to advance and facilitate collaborations.

CORE B: LABORATORY CORE

The main goal of the WIHS Laboratory Core is to ensure efficient and proper collection and use of WIHS specimens. Specific aims can be divided into two main categories (1) QA/methodology and (2) support of WIHS scientific initiatives, and include:

AIMS related to methodology and quality assurance: (1) To ensure WIHS specimens are collected with the least risk to WIHS participants. (2) To delineate collection procedures and an order of collection based on the relative scientific value of specimens while accounting for potential contamination adverse effect(s) incurred during collection of later specimens. (3) To monitor the quantity and type of specimens sent for central long-term storage. (4) To monitor lab quality control activities and certifications.

AIMS in support of WIHS scientific initiatives: (1) To recommend specimens and methods for collection, and tests to be done to fulfill the core aims of WIHS-IV. (2) To ensure that the type and amount of specimens collected, as well as their processing methods, are appropriate and sufficient for proposed scientific investigations. (3) To review new proposals for specimens to be shipped to investigators from the central repositories; to ensure the scientific integrity of each proposal; and to prevent depletion of important repository resources. (4) To work with investigators to compile compatible laboratory data from separate projects into one data set for archive at WDMAC.

CORE D: SOCIAL, BEHAVIORAL, AND SUBSTANCE USE CORE

AIM 1. Engage in interdisciplinary collaborations that serve to integrate social and behavioral science perspectives and methods into WIHS projects by providing ongoing input into study design, implementation, and analysis.

AIM 2. Conduct research on social, psychological, and behavioral factors associated with HIV-related morbidity, mortality, and quality of life, including sexual risk behavior, medication adherence, mental health, substance abuse, and the interrelationships between these factors.

CORE E: LIVER DISEASE AND VIRAL HEPATITIS CORE

AIM 1. To define the hepatitis B and C status of all women in the WIHS cohort by the best available methodologies.

AIM 2. To determine clinical and serum markers that best predict the rate of progression of liver disease in women in WIHS who are co-infected with chronic HCV in order to estimate the fibrosis status of co-infected women over time.

C. WIHS-III CORE AIMS AND HYPOTHESES

The WIHS is a sizable research program designed to directly address key hypotheses related to the epidemiology of HIV among women. For this application, we have organized our research agenda among seven broad specific aims. Each specific aim details “Core” aims & hypotheses that reflect priorities, capabilities, and approaches using the resources provided by this renewal.

The Core aims only partially reflect the strength of the WIHS. The study also serves as a platform for many externally-funded collaborations (e.g., R01s using data and specimens in nested studies within the cohort). These projects rely on the WIHS to carefully collect and store specimens, comprehensively characterize therapy and other exposures, and accurately assess important study endpoints. In return, many of these nested studies contribute important data back to the core (e.g. viral resistance, HPV infection, HCV infection) that is vital for broader investigations. Therefore, for each specific aim, we discuss how existing nested collaborations synergize with the core aims.

Lastly, we have anticipated important research aims and collaborations that would be well-served by WIHS data and specimens, but are not feasible to implement using the resources provided by this renewal. We have identified these as “Platform Aims” and discuss how the WIHS is proactively building partnerships which leverage the established infrastructure to generate broader contributions beyond our Core aims. For background and scientific information for the following, please refer to the WIHS-III grant renewal and Reverse Site Visit presentations.

SPECIFIC AIM 1: MEASURING ART EXPOSURE

AIM 1.1. To develop, refine, and evaluate composite measures of antiretroviral therapy (ART) exposure that comprehensively integrate biological measurements with participants’ self-reported adherence and use of concomitant medications.

AIM 1.2. To relate these measures of antiretroviral therapy exposure to markers of disease progression and adverse effects.

- **Hypothesis 1.2.1.** To assess exposure to antiretroviral treatment including indices of adherence, clearance, and bioavailability. ART exposure will be the most important factor associated with virologic suppression, CD4 cell count trajectory, and the occurrence of adverse effects in the WIHS cohort.
- **Hypothesis 1.2.2.** The principal factors that contribute to ART exposure in WIHS are adherence to the treatment regimen, characteristics of the regimen, pharmacokinetics (ratio of bioavailability to clearance). We hypothesize that variations in pharmacokinetics will contribute as much as adherence to total ART exposure.
- **Hypothesis 1.2.3.** Adverse effects, including elevations of blood triglycerides, will be associated with the highest levels of ART exposure.
- **Hypothesis 1.2.4.** Use of recreational drugs (MDMA, other amphetamines, GBH, ketamine, phencyclidine, heroin, meperidine, methadone, cocaine, marijuana) are common factors that influence exposure to antiretroviral therapies and when used by women who received HAART, will be associated with excess adverse effects.
- **Hypothesis 1.2.5.** To identify the factors that significantly influence variance in bioavailability and clearance of the 2 most commonly used antiretroviral protease inhibitors and the 2 most commonly used non-nucleoside reverse transcriptase inhibitors among women enrolled in the WIHS cohort including use of other drugs and medications, ethnicity, body mass, liver and renal function, diet, symptoms such as diarrhea and fever, smoking and concurrent infections such as hepatitis C.

PLATFORM AIMS:

- To relate measures of antiretroviral therapy exposure to the development of antiretroviral resistance.
- To collect and reposit hair samples collected from each WIHS participant over time to permit for future direct measurement of antiretroviral treatment exposure.

SPECIFIC AIM 2: EFFECTIVENESS OF THERAPY

To define the treated history of HIV infection and the individual determinants (including host and viral genetic factors) of the response to antiretroviral therapy in the era of HAART.

AIM 2.1. To determine the prognostic value of variables measured **before** HAART initiation:

- **Hypothesis 2.1.1.** In the absence of adjustment for time-varying factors, disease progression up to ten years after initiating HAART will be determined by the following characteristics **at the time of HAART initiation**: (a) Presence of clinical disease (i.e., AIDS); (b) Low (<200) CD4+ cell counts, but moderate (200-350) and higher (>350) levels of CD4+ cells at time of initiation will have similar rates of progression; (c) Higher levels of HIV-1 RNA with a dose response relationship. In the presence of time varying predictors (after HAART initiation), only the presence of clinical disease prior to initiating HAART will predict clinical outcome.
- **Hypothesis 2.1.2.** Measures of functional immunologic competence will be associated with disease progression in women with CD4+ cell counts 200-350 cells/ml. For example, diminished responses to delayed type-hypersensitivity testing will be associated with more rapid disease progression and death.

AIM 2.2. To determine if disease progression is predicted best by time-dependent characteristics after HAART initiation:

- **Hypothesis 2.2.1.** Disease progression and death **after** HAART initiation will be predicted by the following time-varying characteristics **measured after initiation of HAART**: (a) CD4+ cell counts as a continuous variable, with a more pronounced effect at CD4<200 cells/ml; (b) Higher levels of HIV-1 RNA with a dose response relationship; (c) continued exposure to ART, as described by the composite measures in Specific Aim 1.
- **Hypothesis 2.2.2.** In participants receiving HAART, and with CD4+ cell counts <200 cells/ μ l or quantitative HIV-1 RNA > 5000 cps/mL, a switch to a new HAART regimen with at least three new drugs will be associated with lower rates of new AIDS defining illnesses and death, compared to women with similar parameters who do not switch HAART regimens.
- **Hypothesis 2.2.3.** Serum albumin and anemia will be independent predictors of clinical outcomes, both in analyses of pre-HAART characteristics, and in analyses utilizing time varying covariates.

AIM 2.3. To determine the association of race with virologic, immunologic and clinical progression (collaboration with MACS):

- **Hypothesis 2.3.1.** Even after adjustment for ART exposure (adherence, changes in therapy, and measures derived in Specific Aim 1) and socioeconomic factors, virologic and immunologic response will be less in African-American and Latina women as compared to Caucasian women.
- **Hypothesis 2.3.2.** In spite of lower viral loads at HAART initiation, African-American women will not experience enhanced virologic suppression, improved CD4+ cell counts, and lower rates of clinical outcomes.
- **Hypothesis 2.3.3.** After accounting for MDR1 and HLA genotype, there will be no difference in the virologic, immunologic, or clinical response to HAART by race/ethnicity.
- **Hypothesis 2.3.4.** After adjusting for race/ethnicity, adherence, changes in therapies, and socioeconomic factors, the relationship of on-HAART (time-varying) HIV RNA with clinical outcomes will be the same in women enrolled in the WIHS and men enrolled in the MACS.

REVERSE SITE VISIT REVISED AIMS:

- To link “population effectiveness” with instrumental variables and implement new measures of effectiveness.

- To explore innovative methods for overcoming selection-by-indication effects for evaluation discontinuation of HAART and continuous changes in markers at HAART initiation.
- To determine if co-receptor genotype influences virologic and immunologic response to HAART.
- To determine the influence of HAART on development and prognosis of HIV related renal disease.
- To evaluate the effects of HIV infection/HAART on:
 - Age of onset of diminished ovarian reserve (increased serum estradiol and FSH and low inhibin B levels at days 2-4 of menstrual cycle).
 - Perimenopausal changes in menstrual cycle characteristics.
 - Age at menopause and menopausal symptoms.
- To determine if thymic output pre-HAART or post-HAART predicts recovery of immune function or sustained suppression of HIV.
- To determine if thymic output predicts immunologic discordant response (substantial viral load decline with no significant CD4 rise).
- To determine potential gender differences in the impact of age on immune recovery after HAART initiation (with MACS).
- To determine potential gender differences in cytokines and the emergence of lipodystrophy.
- To determine if coreceptor utilization varies between blood and genital compartments.
- To determine factors associated with genital tract HIV shedding.
- To determine if a separate reservoir for HIV replication exists among women with suppressed plasma HIV RNA.
- To develop appropriate statistical methods for evaluating heterogeneity in compartmentalization.
- To compare resistance patterns in plasma and genital reservoirs.
- In virologic rebound, does the emergent virus demonstrate both genotypic drug resistance and a preference for X4 use?

PLATFORM AIMS:

- To determine the association of pre-HAART HIV coreceptor utilization (e.g. CCR5/NSI or CXCR4/SI) for predicting response to HAART and coreceptor utilization upon viral rebound.
- To determine the incidence of virologic rebound during HAART therapy and the relative proportion of rebound from wild-type virus versus virus containing resistance mutations.
- To determine the outcomes associated with virologic rebound, with specific emphasis on distinguishing the effects of wild-type versus resistant genotype viral rebound over six months to six years HAART.

SPECIFIC AIM 3: LIPODYSTROPHY IN THE WIHS

To evaluate the effect of HIV and ART on (1) fat redistribution, (2) insulin resistance, glucose intolerance, and diabetes, (3) dyslipidemia (4) cardiovascular disease markers and inflammatory markers of cardiovascular disease and (5) osteopenia and osteoporosis. HIV-uninfected women serve as important control groups, which allow the WIHS cohort to address these aims.

AIM 3.1. To quantify limb fat over time using DXA scans in premenopausal and postmenopausal HIV + and HIV - women, and determine the association with specific antiretroviral drugs, classes of drugs, HAART, HIV infection duration, and menopause. To determine the association of changes in limb fat with specific antiretroviral drugs, classes of drugs, HAART, and with HIV infection itself, CD4, and viral load.

- **Hypothesis 3.1.1.** Limb fat is decreased in HIV+ women due to both HIV infection and antiretroviral therapy.
- **Hypothesis 3.1.2.** Limb fat will be decreased in HIV+ women before the initiation of antiretroviral therapy when compared to HIV- women.
- **Hypothesis 3.1.3.** Limb fat will decrease over time in HIV+ women on antiretroviral therapy.
- **Hypothesis 3.1.4.** Menopausal women not on hormone replacement therapy (HRT) will have higher arm fat but not leg fat than pre-menopausal women.
- **Hypothesis 3.1.5.** Self-report and clinical exam of body fat changes will correlate strongly with quantity of limb fat as measured by DXA in women.

AIM 3.2. To measure truncal obesity over time through the quantification of truncal fat on DXA scans in premenopausal and postmenopausal HIV + and HIV – women. To determine factors associated with truncal obesity, including age, race, CD4, viral load, specific antiretroviral drugs, classes of drugs, and HAART.

- **Hypothesis 3.2.1.** Truncal fat will increase over time in HIV+ women on antiretroviral therapy due, in part, to increases in the number of HIV+ women transitioning to menopause.
- **Hypothesis 3.2.2.** After controlling for menopausal status, truncal fat is decreased in HIV+ women compared to HIV- women.
- **Hypothesis 3.2.3.** After controlling for menopausal status, HAART will lead to increased truncal fat in HIV+ women over time.
- **Hypothesis 3.2.4.** Self report and clinical exam will not accurately estimate truncal fat and both anthropometric measurements and quantification of truncal fat on DXA will be needed to accurately assess abdominal fat gain over time.

AIM 3.3. To measure the prevalence of fasting hyperglycemia, insulin resistance, and diabetes in premenopausal and postmenopausal HIV + and HIV – women. To determine factors associated with disorders in glucose metabolism among HIV infected women, including use of protease inhibitors, and to a lesser extent NRTIs, as well as truncal obesity.

- **Hypothesis 3.3.1.** Fasting hyperglycemia, insulin resistance, and diabetes in HIV+ women will be more prevalent than in HIV- women due to protease inhibitors and to a lesser extent NRTIs, when controlled for truncal obesity and menopause.
- **Hypothesis 3.3.2.** Fasting hyperglycemia, insulin resistance, and diabetes in HIV+ women will be more prevalent in the presence of truncal obesity, when controlled for antiretroviral therapy and menopause.
- **Hypothesis 3.3.3.** Fasting hyperglycemia, insulin resistance, and diabetes in HIV+ women will increase over time due to increases in the number of HIV+ women transitioning to menopause.

AIM 3.4. To measure lipid markers including triglycerides, HDL, direct LDL, and VLDL in premenopausal and postmenopausal HIV+ and HIV- women. To determine factors associated with abnormal lipid measures, including HIV itself, antiretroviral treatment exposure (dosage and interval characteristics, duration of use, constituents of the regimen), truncal obesity, age and race.

- **Hypothesis 3.4.1.** Pro-atherogenic lipid markers will be increased in HIV+ women due to HIV itself, antiretroviral treatment exposure, truncal obesity, and menopause.
- **Hypothesis 3.4.2.** Pro-atherogenic lipid markers will increase in premenopausal women who become menopausal after controlling for treatment exposure effects.

AIM 3.5. To measure the prevalence of the inflammatory marker of cardiovascular disease, C-reactive protein (CRP) in HIV+ and HIV- women. To determine factors associated with abnormal CRP levels, including HIV itself, truncal obesity, menopause and the use of HRT. The contribution of age, race, and smoking will also be assessed.

- **Hypothesis 3.5.1.** CRP levels will be increased in HIV+ women due to HIV itself, antiretroviral therapy, truncal obesity, and menopause.
- **Hypothesis 3.5.2.** When controlling for obesity, menopause will no longer be associated with elevations in C-reactive protein.
- **Hypothesis 3.5.3.** CRP levels will increase in premenopausal women who become menopausal.

AIM 3.6. To measure the prevalence and progression of osteopenia and osteoporosis using DXA scans in premenopausal and postmenopausal HIV+ and HIV- women. To determine risk factors for osteopenia, including abnormal CRP, past use of corticosteroids, smoking, age, race and in HIV+ women, HIV itself and antiretroviral therapy use, classes of antiretroviral drugs, and specific antiretroviral drugs.

- **Hypothesis 3.6.1.** Risk factors for osteopenia include inflammatory markers, past use of corticosteroids, menopause, and in HIV+ women, HIV itself and antiretroviral therapy.
- **Hypothesis 3.6.2.** Peripheral fat loss is associated with osteopenia and osteoporosis.

SPECIFIC AIM 4: ART AND HCV INFECTION

AIM 4.1. To determine the association of HCV infection with response to antiretroviral therapy.

- **Hypothesis 4.1.1.** HIV/HCV co-infected women will have more rapid progression to clinical AIDS and death after HAART initiation compared to HCV-uninfected women, adjusting for HAART exposure.
- **Hypothesis 4.1.2.** HIV/HCV co-infected women will have diminished virologic and immunologic response to HAART compared to HCV-uninfected women, including a diminished CD4+ cell count increase, a faster time to virologic failure, and a higher overall incidence of virologic failure, adjusting for HAART exposure.
- **Hypothesis 4.1.3.** HIV/HCV co-infected women who are able to tolerate long-term anti-HCV therapy will have better virologic and immunologic response to HAART than HIV/HCV co-infected women not on HCV therapy, adjusting for HAART exposure.

AIM 4.2. To evaluate the incidence and predictors of liver injury, dysfunction, and disease (defined below) as a consequence of HIV and HCV infection.

- **Hypothesis 4.2.1.** HIV/HCV co-infected women on HAART will have higher levels of hepatic injury (e.g. higher transaminase levels) and dysfunction markers than HIV/HCV co-infected women not on HAART, and both of these groups will have higher levels than HIV-uninfected women.
- **Hypothesis 4.2.2.** Continued use of alcohol will result in higher incidence of liver disease and liver-related deaths among HIV/HCV co-infected women who are using HAART.

- **Hypothesis 4.2.3.** Increased exposure to certain antiretroviral therapy medications and combinations that include d4T and ritonavir, will lead to more rapid liver disease progression among HIV/HCV co-infected women.

AIM 4.3. To determine the rate and predictors of HCV treatment in HIV/HCV co-infected women. To determine if HCV infection is associated with changes in body fat content and distribution prevalence in women with HIV.

AIM 4.4. To continue to provide an essential platform to support the current WIHS related Hepatitis C R)1s and work with investigators on submissions of R01s to expand this agenda.

AIM 4.5. To examine the effect of alcohol and drug use and treatment on HAART utilization, HAART, effectiveness, and HIV disease progression. To examine the changing substance use patterns in women with and at risk for HIV infection. To examine the effect of substance use and its treatment on health care utilization and mental illness and treatment. To examine the relationships of substance use, violence history and HIV therapy effectiveness.

AIM 4.6. To define the clinical significance of isolated Hepatitis B Core Antibody positivity.

AIM 4.7. To determine the relationship of cigarette smoking on HPV.

PLATFORM AIMS:

- Investigate whether HCV viral load, genotype, and quasi-species diversity influence HIV-1 viral load, CD4 decline, functional immunity (naïve and memory subsets of CD4 and CD8 cells) and response to HAART. Determine the prevalence of extrahepatic HCV replication among co-infected women and assess its relationship with HIV viral load, CD4 count, and disease progression.
- Determine whether level of HCV viremia and the presence of extrahepatic replication in peripheral blood mononuclear cells (PBMC) are associated with increased immune activation, alterations in naive and memory CD4 cell numbers and thymic function (T cell receptor excision circles (TREC) concentrations).
- Investigate the association between HCV viremia, genotype and quasi-species diversity on progression of liver disease and response to HCV-specific therapy in HIV infected women
- Determine level of HCV viremia and incidence of extrahepatic replication in PBMCs among HIV+ women with acute HCV infection and assess for impact on immune and thymic function.
- Assess for HCV RNA negative strand and viral quasispecies composition in serum, PBMC, and CVL in HIV coinfecting patients and HIV-negative HCV+ women.
- Compare HCV and HIV-1 viral loads in peripheral blood, oral and cervicovaginal mucosal sites in the presence and absence of immune reconstitution following initiation of HAART and to determine if HAART influences quasispecies composition in these sites.
- Study HIV viral dynamics following HAART in the presence or absence of HCV infection and assess for associated changes in T cell populations, thymic function, and immune function in a subset of intensively followed women.
- Identify the type of cells present in genital secretions (CVLs) both in HIV-1 infected and uninfected patients co-infected with HCV and to characterize HCV viral strains/quasispecies in blood and CVL using sequencing and in-vitro infection culture techniques.
- Determine the similarity between sequences of HCV 5'NTR from CVL and peripheral blood from the same HIV-1 infected subject and correlate differences with treatment, route of infection and history of active drug use.

- Evaluate the role of host genetics (HLA class I and II genotype) as a co-factor for HIV disease progression, development of liver disease and response to HCV therapy among HIV/HCV co-infected women. Determine if HCV+/HIV+ women have an increased prevalence of immune-mediated and lymphoproliferative disorders.

SPECIFIC AIM 5: EFFECTS OF AGE, MENOPAUSE AND ITS TREATMENT ON THE COURSE OF HIV INFECTION

AIM 5.1. To evaluate the effects of age, menopause and its treatment on the course of HIV infection and the virologic and immunologic response to antiretroviral treatment.

- **Hypothesis 5.1.1.** Older women (particularly those older than 45) will have a greater incidence of new AIDS defining conditions after HAART initiation as compared with younger women, adjusting for HAART drug exposure.
- **Hypothesis 5.1.2.** After accounting for thymic function (measured using T-cell excision circle, TREC) concentrations in multivariable analysis, older women will no longer have a higher risk of AIDS after HAART initiation.
- **Hypothesis 5.1.3.** HIV-infected menopausal women (defined as one year without the occurrence of a menstrual period among women with no known extraneous cause for amenorrhea) will have improved thymopoiesis relative to HIV-infected premenopausal women as evidenced by an increased percentage of naïve CD4 cells and higher TREC concentrations (controlling for HAART exposure).
- **Hypothesis 5.1.4.** After HAART initiation, and controlling for HAART exposure, menopausal HIV-infected women reporting use of hormone replacement therapy (HRT) will have more consistent virologic suppression but smaller increases in CD4 cell count and TREC concentration, than menopausal HAART recipients who have not received HRT.

AIM 5.2. To evaluate the effects of HIV infection on the age of onset of diminished ovarian reserve (increased serum estradiol and FSH and low inhibin B levels at days 2- 4 of menstrual cycle), perimenopausal changes in menstrual cycle characteristics, and the age at menopause.

- **Hypothesis 5.2.1.** HIV infected women will have evidence of diminished ovarian reserve and irregular menstrual cycles at earlier ages than HIV uninfected women.
- **Hypothesis 5.2.2.** HIV infected women will transition to menopause at an earlier age than HIV-uninfected women.
- **Hypothesis 5.2.3.** In the absence of HRT, treatment with protease inhibitor regimens will be associated with earlier perimenopausal symptoms and increased FSH levels.
- **Hypothesis 5.2.4.** Increased FSH and diminished ovarian function will be associated with reduced fertility and increased risk of miscarriage when controlled for age.

AIM 5.3. To evaluate the effect of endogenous and exogenous hormones on circulating RANTES and MCP-1 levels and their association with plasma HIV RNA level.

- **Hypothesis 5.3.1.** Among premenopausal and HRT-free women, RANTES and MCP-1 levels will be higher in women with a prior history of endometriosis as compared to women with no history of endometriosis.
- **Hypothesis 5.3.2.** Among premenopausal and HRT-free women, HIV RNA levels, RANTES and MCP-1 levels will be highest during the midluteal phase of the ovulatory cycle.
- **Hypothesis 5.3.3.** RANTES and MCP-1 levels will be lower in women with a hysterectomy or menopause not reporting HRT as compared to premenopausal ovulating women.

- **Hypothesis 5.3.4.** RANTES and MCP-1 levels will be higher in menopausal women reporting HRT, but less than premenopausal ovulating women during the midluteal phase.
- **Hypothesis 5.3.5.** During pregnancy, RANTES and MCP-1 levels will positively correlate with increased serum progesterone levels.

SPECIFIC AIM 6: CANCER LESIONS AND CANCER INCIDENCE

AIM 6.1. To study the long term effects of HIV infection and use of HAART on the natural history of HPV and cervical dysplasia.

- **Hypothesis 6.1.1.** The incidence of severe cervical dysplasia will be increased in the presence of HIV-infection and in the presence of low (<200/dL) CD4+ T-cell levels.
- **Hypothesis 6.1.2.** Among HIV-infected women, use of HAART will be associated with reduced rates of severe cervical dysplasia, although these rates will be elevated relative to those in HIV-uninfected women. Because of improved survival (but still diminished immunity) in HIV+ women, the cumulative incidence of severe cervical dysplasia will increase rather than decrease during the HAART era.

PLATFORM SPECIFIC AIMS: HPV AND CERVICAL NEOPLASIA

- To study the effects of HPV intratype variation on the natural history of representative oncogenic (HPV16, 18, 31, 58) and non-oncogenic (HPV 6 and 53) HPV types in HIV-infected and -uninfected women.
- To examine complete high resolution HLA class I and II genotype and its relation to the natural history of HPV and cervical dysplasia in HIV-infected and -uninfected women.
- To determine whether HPV can become latent and then be reactivated in HIV-infected women, by detection of the same HPV type-specific variant at two time points that are separated by sequential negative specimens (focusing on women who reported being sexually inactive for that entire period).
- To study humoral immune responses to HPV and their relation with the natural history of HPV infection and development of cervical disease in HIV-infected and -uninfected women.
- To determine whether development of HPV type-specific antibodies protects against subsequent infection with the same HPV type.
- To study the natural history of anal HPV infection and anal neoplasia among HIV-infected and high-risk HIV-uninfected women in the HAART era.
- To compare the natural history and risk factors for anal versus cervical HPV infection and cytologic abnormalities among HIV-infected and high risk HIV-uninfected women, including comparison of the intratype HPV variants of HPV 16, 18 and 31 in the anus and cervix.

AIM 6.2. To determine if there is an increased incidence of specific cancers among HIV-infected women as compared to HIV-uninfected women, to the general population (SEER), to the women in the Veterans Administration database; and to HIV infected men in the MACS.

- **Hypothesis 6.2.1.** The incidence of AIDS-related cancer in both pre- and post-HAART eras will be higher than expected among HIV-infected women, when compared to age- and race-adjusted HIV-uninfected WIHS women and to the age- and race-adjusted general population.
- **Hypothesis 6.2.2.** The incidence of certain non-AIDS-related cancers (such as lung cancer) will be higher than expected among HIV-infected and HIV-uninfected WIHS women, when compared to age- and race-adjusted rates in the general population.

PLATFORM AIM: NON-CERVICAL MALIGNANCIES

- To provide access to state-of-the-art therapies to HIV infected women with malignant disease.

AIM 6.3. To evaluate the impact of HAART on HIV- and non-HIV-associated cancers in HIV-infected women.

- **Hypothesis 6.3.1.** The use of HAART, and response to HAART, will be associated with a decreased incidence of AIDS-related cancers and a longer survival time with cancer in HIV-infected participants.
- **Hypothesis 6.3.2.** The use of HAART, and response to HAART, will be associated with a change in the clinical presentation of both HIV- and non-HIV-associated malignancies.

AIM 6.4. To investigate the immunologic, virologic, genetic and environmental risk factors for the most commonly occurring cancers in the WIHS (NHL, KS, cervical and lung), .

- **Hypothesis 6.4.1.** The excess incidence of lung cancer will be attributed to cigarette smoking and not HIV-infection. However, lung cancers in HIV-infected women will occur at an earlier age and characteristics of disease will differ from lung cancers in HIV-uninfected women.
- **Hypothesis 6.4.2.** The incidence of KS will be associated with HIV-infection and HHV-8 infection, as well as other markers of immune suppression.
- **Hypothesis 6.4.3.** The incidence of NHL will be associated with HIV-infection, as well as other markers of immune suppression.

AIM 6.5. To collect fresh-frozen tissue from biopsies of the cervix, vagina, and vulva and accompanying blood specimens for donation to the NCI-sponsored AIDS Cancer and Specimen Bank (ACSB).

PLATFORM AIM: NON-CERVICAL MALIGNANCIES

- To determine the role of genetic polymorphisms in the IL-10 promoter genes in predicting the development of non-Hodgkin's lymphoma among HIV infected women, and whether serum IL-10 levels will be increased in HIV infected women with IL-10 promoter polymorphisms prior to the onset of AIDS lymphoma

AIM 6.6. To determine if enhanced B cell stimulation (elevated serum levels of B cell-stimulatory cytokines or of immune system molecules associated with B cell activation) and/or increased in vivo EBV levels precede the development of AIDS-NHL. To determine if HAART affects the pre-lymphoma expression of B cell stimulatory molecules and/or in vivo EBV levels. To determine if polymorphisms in the genes encoding B cell-stimulatory cytokines or cytokine receptors are associated with an elevated risk for the development of AIDS-NHL.

SPECIFIC AIM 7: ORAL BIOLOGY

AIM 7. To evaluate the oral manifestations of HIV disease in women using the well-defined cohort of women enrolled in the WIHS Oral Protocol.

- **Hypothesis 7.1.** A decrease in the prevalence and incidence of oral disease is associated with HAART and an increase is associated with HAART failure
- **Hypothesis 7.2.** There are oral adverse effects related to HAART therapy including an increased incidence of oral warts, salivary gland disease and caries and a decrease in salivary flow.
- **Hypothesis 7.3.** There is a difference in the periodontal disease experience between HIV positive HAART naive, HIV positive on HAART and HIV negative women.

- **Hypothesis 7.4.** There is pathogenic synergy between viruses prevalent in HIV positive women and the bacteria implicated in periodontal disease.
- **Hypothesis 7.5.** There is a difference in caries prevalence/incidence in HIV positive HAART naïve, HIV positive on HAART and HIV negative women.

D. WIHS-II CORE RESEARCH QUESTIONS

Key areas of investigation may include:

1. Characterization of women-specific outcomes among HIV-infected women, including genital infections, sexually transmitted diseases and uterine cervical abnormalities.
2. Impact of acute clinical events or concomitant infections on HIV viral load and disease progression.
3. Interaction between pregnancy and HIV infection (in collaboration with Women-Infant Transmission Study).
4. Impact of antiretroviral treatment (and resistance to antiretrovirals) on the pattern of HIV disease progression in infected women.
5. Treatment compliance and its relationship with acute clinical events.
6. Surveillance, risk factors (immunological, virological, genetic and environmental), molecular epidemiology and natural history of HIV-associated cancers and their etiologic precursors in women.
7. Impact of endocrinologic factors (endogenous and exogenous hormonal) on:
 - 1) Mucosal immunity and HIV expression in the female genital tract, and
 - 2) Co-infections in the female genital tract and HIV disease progression in women.
8. Epidemiology and molecular mechanisms underlying HIV-associated oral manifestations and characterization of saliva as a source of HIV inhibitory factors, a vehicle for drug delivery and for HIV diagnostic tests.
9. Substance abuse, sexual behaviors, psychosocial outcomes and their relationship with the design of risk reduction interventions.
10. Impact of continuing substance abuse on virologic and immunologic status and treatment effectiveness.
11. Markers of high-risk behavior (including commercial sex and exchange of sex for drugs).
12. Healthcare utilization and barriers among women with HIV.
13. Innovative methodological approaches to cohort studies of HIV/AIDS.
14. Collaborative studies with other cohorts on HIV/AIDS.

1. SITE-SPECIFIC CORE RESEARCH QUESTIONS – Bronx/Manhattan

1. To determine the rate of progression of HIV-associated disease in both symptomatic and asymptomatic women and to correlate progression with multiple parameters, including:
 - a. viral burden
 - b. immunological parameters

- c. use of HAART, alternative therapies, prophylaxis for OIs, antibiotics and antivirals (for agents other than HIV)
 - d. exogenous hormone use
 - e. coinfection with HTLV-I and HTLV-II
 - f. coinfection with TB
 - g. behavioral cofactors, including sustained antigenic exposure (injection drug use, unsafe sex)
2. To determine incidence, prevalence, risk factors associated with development of AIDS-defining infections, malignancies and other conditions, both definitely and presumptively diagnosed.
 3. To define survival in the WIHS cohort from time of AIDS diagnosis, HIV-associated illnesses, ranges and progression of CD4 counts, quintiles of quantitative RNA at study entry; and to determine the association of survival with HAART, prophylaxis for opportunistic illness, compliance with prescribed therapies, exposure route, continued antigenic stimulation, race/ethnicity and CCR-5 receptor status.
 4. To assess compliance with HAART and the demographic, cultural and behavioral factors associated with non-compliance.
 5. To determine prevalence, incidence and factors including HAART and quantitative plasma HIV RNA associated with genital SIL.
 6. To determine rate of expression of HPV and its subtypes in the cervicovaginal secretions of HIV-infected and uninfected women.
 7. To determine if there is down regulation of HIV expression in the genital tract of HIV infected women receiving HAART.
 8. To determine the level of quantitative HIV RNA in the genital tract and its correlation with clinical and hormonal status, vaginal coinfections, local virologic and immunologic parameters and the level of HIV RNA in plasma, both in the untreated state and in response to HAART.
 9. To evaluate the effect of HIV-related immunosuppression on the incidence, prevalence, natural history of genital infections.
 10. To determine the access of HIV infected women to HAART.
 11. To determine the effect of HIV infection and HIV-related therapy on the menstrual cycle.
 12. Specific hypotheses in WIHS-II:
 - a. In the area of disease progression
 - b. In gynecologic disease and infection
 - c. In obstetrical studies
 - d. In malignancy studies
 - e. In investigations of behavioral parameters and of health care utilization
 - f. In study of emergence of resistance to HAART
 - g. In investigations of host factors associated with resistance to infection and with delayed disease progression

2. SITE-SPECIFIC CORE RESEARCH QUESTIONS – Brooklyn

1. Progression (natural history) and emergence of resistance protocol
 - **Hypothesis A.** In the era of new antiretroviral agents, viral load, virus type, immune markers, coinfections, vitamin A levels, specific sexual and drug use behaviors, socio-economic status, social support and receipt of treatment will predict subsequent rate of CD4 decline and AIDS-free survival.
 - **Hypothesis B.** Viral load will predict incidence of opportunistic infection, wasting and time to death in women with < 50 CD4 cells.
 - **Hypothesis C.** The course of HIV infection and progression will differ in women who acquire infection sexually (mucosally) vs. parenterally; and ongoing exposure to drugs will affect the course of disease.
 - **Hypothesis D.** Genetic factors (e.g., CCR-5 receptor status) correlate with the rate of disease progression.
 - **Hypothesis E.** Drug regimen, drug levels, compliance, viral load and genetic factors will predict the development of resistance and the presence of resistance will predict more rapid disease progression.
2. Genital tract neoplasia and related infections protocol (gynecological protocol)
 - **Hypothesis A.** The prevalence and incidence of genital squamous intraepithelial lesions correlates with CDC stage of HIV disease, HPV infection and type, HIV load, antiretroviral therapy, interleukins and rate of CD4 depletion.
 - **Hypothesis B.** Endocrinopathies occur at an increased rate among women with advanced immune compromise.
 - **Hypothesis C.** The local immunologic milieu and ecosystem in cervical/vaginal tissues of HIV-infected women predicts HIV disease progression and predicts HIV shedding in CVL samples.
3. Malignancy protocol
 - **Hypothesis A.** HIV-seropositive women in the WIHS will have an increased incidence of cancer over time, when compared to HIV-seronegative controls.
 - **Hypothesis B.** Immune deficiency predisposes to the development of neoplastic disease, including unusual pathologic types of malignant disease of the breast.
 - **Hypothesis C.** HIV-infected women who are coinfecting with KS-associated Herpes virus (HHV-8) are at increased risk for development of Kaposi Sarcoma and/or certain types of B cell lymphoma (NCI-funded).
 - **Hypothesis D.** Coinfection with HTLV-I and HTLV-II is more common in HIV-infected women who acquire HIV infection through injection drug use than by sexual (mucosal) means and correlates with development of malignant disease over time, including occurrence of adult T-cell leukemia/lymphoma (ATLL).
4. Behavioral protocol
 - **Hypothesis A.** The level of depression and quality of life among HIV-infected women will vary over time with their illness course and symptom level.
 - **Hypothesis B.** Prevalence of domestic violence and sexual abuse in the WIHS cohort will be interrelated with a history of substance use.

- **Hypothesis C.** Greater utilization of primary care services is associated with greater use of antiviral and prophylactic therapy, a lower incidence of preventable infections.
 - **Hypothesis D.** Women with dependent children demonstrate less utilization of health care and those with ill children have the highest utilization of care and highest incidence of preventable conditions and depression.
 - **Hypothesis E.** Income and educational achievement are directly correlated with utilization of primary care services, antiviral treatment, and functional status.
 - **Hypothesis F.** High-risk sexual behavior is associated with use of injection and other substance use, lower income and fewer primary care visits.
5. Lower genital tract virology/reproductive health, including pregnancy
- **Hypothesis A.** Pregnancy alters the natural history of HIV disease, starting in the first trimester.
 - **Hypothesis B.** The placenta serves as a barrier to virus and drugs during pregnancy.
 - **Hypothesis C.** Pregnancy leads to an up regulation of coincident infections and changes the consequences thereof.
 - **Hypothesis D.** In-utero exposure to antiretroviral therapy may affect the risk of neoplasia in children.
 - **Hypothesis E.** High maternal viral load will correlate with early pregnancy wastage and perinatal transmission of other pathogens.
 - **Hypothesis F.** The presence of HIV in CVL samples correlates with clinical status, hormonal changes, antiretroviral therapy, microbicide use and local virologic and immunologic parameters, and vaginal coinfections.
 - **Hypothesis G.** (1) Correlations will exist between HIV viral load in CVL samples and in the peripheral blood. (2) The presence and titer of specific antibody (systemic or local) will influence the type and quantity of virus isolated from CVL. (3) The presence of coinfections and/or hormonal factors will influence the quantity and type of virus isolated from CVL.
6. Seronegative protocol
- **Hypothesis A.** Immune response to HIV will differ between exposed-uninfected, exposed-infected and unexposed individuals.
7. Progression and emergence of resistance protocol
- a. The relationship of HIV-1 type (e.g., multiply spliced vs. unspliced) to disease progression.
 - b. The relationship between vitamin A levels and disease progression.
 - c. CCR-5 genotypes: relation to HIV transmission and disease progression in the WIHS cohort.
 - d. Modulation of Fc gamma and Fc epsilon receptors on leukocytes during progression of HIV disease.
 - e. Hepatitis C and HIV disease progression.
 - f. Acute clinical events and HIV load.

8. Genital tract neoplasia and related infections protocol
 - a. The relationship of cervical disease to the use of new antiretroviral therapies.
 - b. The relationship of humoral immunity to HPV.
 - c. Dendritic Langerhans cells in the cervix of HIV-infected women.
 - d. Expression of HIV and iNOS in monocyte-derived macrophages and CVL cells.
 - e. HPV and HIV gene expression in cervical neoplasia.
 - f. Cytokine production patterns in CIN in HIV-infected women.
 - g. Vaginal ecosystem and HIV in the female genital tract.
9. Malignancy protocol
 - a. Collaboration with AIDS Malignancy Consortium.
 - b. Breast cancer study.
 - c. Incidence, prevalence and consequences of HHV-8.
10. Behaviors
 - a. Description of marijuana use in a cohort of HIV-negative women.
11. Lower genital tract virology/reproductive health including pregnancy
 - a. Genital tract virology:
 - 1) DATRI 009 and 009a
 - 2) DATRI 009b
 - 3) WHS 001
 - 4) WHS 002
 - b. Pregnancy studies
 - 1) Placental barriers to HIV
 - 2) HPV load and cervical disease in pregnancy
 - 3) Viral load in pregnancy
 - 4) Does pregnancy increase the risk of seroconversion?
 - c. Endocrine studies
 - 1) Adrenal hormones and immune function in women with HIV
 - 2) The effect of contraceptives on vaginal HIV virus
12. HIV seronegative protocol
 - a. The immunologic profile of high-risk exposed seronegative individuals.

3. SITE-SPECIFIC CORE RESEARCH QUESTIONS – Washington, D.C.

1. Core research goals
 - a. Define characteristics that predict HIV disease progression in a representative cohort of HIV-infected women.

- b. Define gender-specific characteristics of HIV disease in women and the association between those characteristics and subsequent HIV disease progression.
 - c. Define the role of co-infecting organisms on HIV disease progression in a representative cohort of HIV-infected women.
 - d. Identify the impact of HIV disease on the psychological and social status of women, including quality of life, depression, neuropsychological functioning and family (partner/child) interactions.
 - e. Characterize factors that may interact to influence experience of HIV-infected women in terms of their access to care, compliance with treatment and psychological and social outcomes.
 - f. Develop methods to increase the enrollment and retention of women, ethnic minorities, and socially disenfranchised individuals in clinical research.
2. Proposed WIHS-II
- a. Natural History – Disease progression in WIHS-II
 - 1) Acute seroconversion
 - 2) Protease inhibitor non-responders (WIHS collaborative)
 - 3) Intercurrent illness (WIHS collaborative)
 - 4) Vaginal and secretory immune response to *Candida albicans*
 - 5) Nutritional assessment in women infected with HIV
 - 6) Immune response in exposed-uninfected (core)
 - 7) WIHS/AIDS malignancy bank (core)
 - b. Gynecologic agenda for WIHS-II
 - 1) Obstetrical protocol (core)
 - 2) CIN and HPV in the pregnant cohort (WIHS collaborative)
 - 3) Mammography (WIHS collaborative)
 - 4) CIN treatment trial (WIHS collaborative)
 - 5) Impact of hormonal therapy on progression of HIV disease (WIHS collaborative)
 - c. Behavior science plan for WIHS-II
 - 1) Depression and patient outcomes
 - 2) Validation of the HIV health survey
 - d. Laboratory agenda for WIHS-II
 - 1) Evaluation of markers of immune activation, function and maturation in HIV disease progress (WIHS collaborative)
 - 2) CCR-5 genotypes: relation to HIV transmission and disease progression in the WIHS cohort (WIHS collaborative)
 - 3) HIV p24 antibody levels as a predictor of disease progression and death on infected women in the WIHS cohort (WIHS collaborative)

- 4) Quantitative HIV-1 RNA determinations on filter paper adsorbed whole blood and cervical vaginal lavage fluids (WIHS collaborative)
- 5) NIDA study immunology (WIHS collaborative)
- e. Lipoproteins in HIV (to evaluate recent findings of large increases in individuals taking protease inhibitors)

4. SITE-SPECIFIC CORE RESEARCH QUESTIONS – Southern California

1. Define characteristics that predict HIV disease progression in a representative cohort of HIV-infected women.
 - **Hypothesis A.** The viral load, immune markers, co-infections, ethnicity, specific sexual and drug use behaviors, socio-economic status, social support and receipt of treatment will predict subsequent rate of CD4 decline and AIDS-free survival.
 - **Hypothesis B.** A threshold level of HIV RNA will predict AIDS progression before CD4 decline.
 - **Hypothesis C.** Viral load will predict incidence of opportunistic infection, wasting and time to death in women with < 50 CD4 cells.
 - **Hypothesis D.** The course of HIV infection and progression will differ in women who acquire infection sexually (mucosally) vs. parenterally; and the course of infection and disease progression will differ in women who are continuously exposed to antigen stimulation and immune activation of injecting or non-injecting drugs as compared to women who have not used these drugs. (NIDA study: “HIV disease in drug-using women: virologic and immunologic predictors of disease progression.”)
2. Define the gender-specific characteristics of HIV disease in women and their association with subsequent HIV disease progression.
 - **Hypothesis A.** The prevalence and incidence of genital squamous intraepithelial lesions correlates with CDC stage of HIV disease, HPV infection and type, HIV load and rate of CD4 depletion.
 - **Hypothesis B.** The presence of HIV in CVL samples correlates with clinical status, hormonal status, anti-retroviral therapy and local virologic and immunologic parameters, and vaginal coinfections.
 - **Hypothesis C.** The rate of menstrual dysfunction is related to immune status.
 - **Hypothesis D.** Endocrinopathies occur at an increased rate among women with advanced immune compromise.
 - **Hypothesis E.** Advanced HIV disease is associated with subfertility.
 - **Hypothesis F.** Virologic and immunologic markers of disease progression are impacted by multiple factors, including hormonal changes, hormonal characteristics, drug use and exposure to multiple infectious agents.
 - **Hypothesis G.** The quantity and character of maternal HIV in plasma and CVL are related to the occurrence of mother-to-child transmission.
 - **Hypothesis H.** High maternal viral load will correlate with early pregnancy wastage and perinatal transmission of other pathogens.
3. Define the role of coinfecting organisms on HIV disease progression in a representative cohort of HIV-infected women.

- **Hypothesis A.** The prevalence and incidence of genital epithelial dysplasias is positively correlated with CDC stage of HIV disease, HPV infection and type, HIV load and rate of CD4 depletion (NCI-funded).
 - **Hypothesis B.** The incidence of oral leukoplakia (EBV infection), oral candidiasis, gingivitis, oral condylomata, oral ulcerative lesions and oral masses is correlated with absolute CD4 count and the occurrence of these conditions is predictive of a higher rate of CD4 depletion.
 - **Hypothesis C.** Coinfection with HTLV-I and HTLV-II is more common in HIV-infected women who acquire HIV infection through injection drug use than by sexual (mucosal) means and correlates with development of malignant disease over time, including occurrence of adult T-cell leukemia/lymphoma (ATLL).
 - **Hypothesis D.** HIV-infected women who are coinfecting with KS-associated Herpes virus (HHV-8) are at increased risk for development of Kaposi Sarcoma and/or certain types of B cell lymphoma (NCI-funded).
4. Identify the impact of HIV disease on the psychological and social status of women, including quality of life, depression, neuropsychological functioning and family (partner/child) interactions.
- **Hypothesis A.** The level of depression among HIV-infected women is elevated over the general population but not over a well-matched control group; quality of life is lower.
 - **Hypothesis B.** Prevalence of domestic violence and sexual abuse in the WIHS cohort is elevated, especially among women with history of substance use.
 - **Hypothesis C.** Prevalence of neuropsychological complications is higher in infected women than that reported in infected men.
5. Characterize factors that may interact to influence experience of HIV-infected women in terms of their access to care, compliance with treatment and psychosocial and social outcomes.
- **Hypothesis A.** Greater utilization of primary care services is associated with greater use of antiviral and prophylactic therapy, a lower incidence and prevalence of preventable opportunistic infections; and few unplanned pregnancies.
 - **Hypothesis B.** Women with dependent children demonstrate lesser utilization of health care than women without dependent children and those with children who are ill have the lowest utilization of care and highest incidence of preventable conditions.
 - **Hypothesis C.** Income and educational achievement are directly correlated with utilization of primary care services, antiviral treatment, preventive regimens, use of unorthodox treatment and functional status, and inversely correlated with occurrence of preventable conditions and emergency treatment.
 - **Hypothesis D.** High-risk sexual behavior is associated with use of injection and other substance use, lower income and fewer primary care visits.
 - **Hypothesis E.** Injection and other drug use interferes with utilization of health care services and medication adherence behaviors of HIV-infected women. (NIDA substudy: “Effect of drug use on HIV-related health care behaviors and adherence to treatment regimens.”)

6. Define the oral manifestations of HIV infection in women.

5. SITE-SPECIFIC CORE RESEARCH QUESTIONS – Northern California

1. Progression
2. Emergence of resistance
3. Genital tract neoplasia and HPV infection
4. Lower genital tract virology/reproductive health
5. Concurrent conditions
6. Malignancies and related infections
7. Studies of the oral cavity
8. HIV-seronegative participants
9. Behavioral issues

6. SITE-SPECIFIC CORE RESEARCH QUESTIONS – Chicago

1. Define characteristics that predict HIV disease progression in a representative cohort of HIV-infected women.
2. Determine the rate of emergence of protease-inhibitor resistance and the association of resistance with adherence to the therapeutic regimen and plasma drug levels.
3. Define the gender-specific characteristics of HIV disease in women and the association between those characteristics and subsequent HIV disease progression.
4. Define the effect of early pregnancy on HIV disease and other co-incident infections.
5. Define the role of malignancy-associated infectious agents and malignancies on HIV disease progression in a representative cohort of HIV-infected women.
6. Define the correlation between HIV disease progression and oral health.
7. Identify the impact of HIV disease on the psychological and social status of women, including quality of life, depression, neuropsychological functioning and family (partner/child) interactions.
8. Characterize factors that may interact to influence experience of HIV-infected women in terms of their access to care, compliance with treatment and psychological and social outcomes.
9. Identify activities, attitudes and immune responses which lead at-risk women in the WIHS seronegative control group to seroconvert while others do not.
10. Develop methods to increase the enrollment and retention of women, ethnic minorities, and socially disenfranchised individuals in clinical research.

E. WIHS-I CORE RESEARCH QUESTIONS

1. Non-Gynecological Natural History:
 - a. To determine the spectrum and course of the clinical manifestations of HIV infection in women, including the incidence and prevalence of:
 - 1) AIDS-defining infections, malignancies and other conditions, both definitive and presumptive diagnoses, as defined by the expanded (1993) CDC definition;
 - 2) Illnesses likely or possibly associated with HIV immune suppression or risk behavior that do not meet CDC criteria, infections, neutropenia and immune thrombocytopenia, herpes

zoster infection, peripheral neuropathy, dermatologic abnormalities, psychiatric disturbances and oral pathology, including oral hairy leukoplakia, candidiasis, thrush, and periodontal disease.

- b. To investigate median survival from:
 - 1) Time of AIDS diagnosis to death.
 - 2) Time of HIV-associated illness to death.
 - 3) Ranges and progression of CD4 cell counts to death; and to determine what factors are associated with or predictive of long-term survival with high CD4 cell counts and low CD4 cell counts (age, HLA, secretor status, race, behavioral risk factors prior to infection, behavioral characteristics subsequent to infection, treatment, location of residence, and viral genetics).
 - c. To determine what factors are associated with or predictive of clinical disease progression (HIV risk behavior, drug use, race, socio-economic factors, use of HIV antivirals and prophylactic therapies, age, HLA).
 - d. To determine virologic markers associated with or predictive of clinical progression or failure to progress (viral induction of syncytia, viral quantitation, P24 antigen).
 - e. To investigate immunologic markers of disease progression including pattern and rate of change of CD4 cells, and other immune markers such as beta 2 microglobulin, and neopterin; and to determine the relationship of the changes to the clinical manifestations of HIV infection.
 - 1) To determine what factors are associated with or predictive of changes in immunologic markers, including: HIV risk behavior, drug use, race, socioeconomic factors, use of HIV antivirals and prophylactic therapies, age, HLA, Haplotype, etc.).
2. Gynecological Natural History
- a. Cervical and anogenital neoplasia: To evaluate the effect of HIV disease on the incidence and natural history of intraepithelial neoplasia and HPV mediated cervical disease:
 - 1) To determine the incidence and prevalence of HPV in HIV-infected and uninfected women;
 - 2) To assess alterations in the course of HPV disease with progressive immunocompromise among HIV-infected women;
 - 3) To compare the natural history and response to therapy of HPV in HIV-infected and HIV-uninfected women;
 - 4) To describe what HPV types are associated with cervical neoplasia in HIV-infected and HIV-uninfected women.
 - b. Genital Infections: To evaluate the effect of HIV disease and HIV immune suppression on the incidence, prevalence, natural history and response to therapy of vaginal candidiasis, Chlamydia, Trichomonas vaginalis, syphilis, pelvic inflammatory disease, bacterial vaginosis.
 - c. To determine the effect of HIV infection and HIV-related therapy on the menstrual cycle.
 - d. To determine the most common vaginal microflora (including aerobic and anaerobic bacteria and fungi) among HIV-seropositive and high-risk seronegative women, and its association with HIV immune suppression.

- e. To determine the incidence and prevalence of hysterectomy among HIV-seropositive and seronegative women and the relationship between stage of HIV-related illness and the incidence of hysterectomy.
3. HIV Seroconversion:
 - a. To determine the rate of incident HIV seroconversion among a cohort of HIV-uninfected high-risk women.
 - b. To describe factors that may influence the risk of HIV seroconversion (such as age, race, socio-economic status, education, sexual practices, contraception, menstrual pattern, pregnancy, vulvovaginal disease, PID, cervical disease, sexually transmitted diseases, injecting and non-injecting drug use, other substance use, domestic violence and homelessness).
 4. Psychosocial/Functional Status:
 - a. To determine the interrelationships among medical interventions, disease progression, intensity of service utilization, with duration and quality of life.
 - b. To determine how the social support system/network moderates the relationship between illness and treatment related factors and quality of life and length of survival.
 - c. To determine the relationship between various symptoms associated with HIV-infection and the occurrence of functional disabilities (including standard measures of functional status and measures specifically adapted for women); and to determine what factors are associated with or predictive of various kinds of symptoms or functional disabilities, including disease stage, prognostic indicators, drug use, treatment utilization, social support and circumstances of living.
 - d. To determine the prevalence of a history of child sexual abuse among HIV-infected and high-risk uninfected women.
 - e. To define the economic impact over time for HIV-infected women compared with HIV-uninfected women.
 - f. How does the course of substance use (alcohol, tobacco, and recreational drugs), change over time among seropositive, and high-risk seronegative women. Is identification of HIV infection, provision of medical care and follow-up associated with an increased or decreased use of various substances? Is clinical progression associated with increased or decreased use of various substances?

F. CORE HERS/WIHS RESEARCH QUESTIONS

1. What are the types, incidences and predictors of AIDS-defining and HIV-related conditions and other clinical conditions?
2. What are the predictors of incidence and clinical course of cervical neoplasia and HPV-mediated cervical disease?
3. What are the predictors of incidence, prevalence and course of disease of genital infections?
4. Are there different trends over time in substance use according to HIV serostatus? according to immune status among HIV-seropositive women?
5. Are there different trends over time in sexual behavior according to HIV serostatus?
6. Are there different trends over time in psychosocial outcomes according to HIV serostatus?
7. Are there differences in mortality according to HIV serostatus?

8. Among HIV-seropositive women, what are the predictors of clinical disease progression as defined by onset of AIDS-defining or HIV-related conditions?
9. Among HIV-seropositive women, what are the predictors of length of survival from AIDS diagnosis? from HIV-associated illnesses? from CD4 thresholds?
10. Among HIV-seropositive women, what is the rate of change and what are the predictors of rate of change of immunologic markers (CD4, CD8, neopterin, Beta2M)?
11. What is the incidence of HIV seroconversion in our sample of HIV-seronegative women?