MWCCS Unified Scientific Agenda

The combined cohort study provides an opportunity to advance knowledge of the basic science, clinical science, and epidemiology of HIV infection in the US, with a focus on HIV-related comorbidities. Building on the current successful MACS and WIHS cohorts we will continue to follow the established group of persons living with HIV infection (PLWH) and risk matched HIV-uninfected already in study follow-up, and will recruit new individuals in high-priorities areas including black and Hispanic MSM, MSM in the southern US, recently infected PLWH, and participants who replace those who are deceased or lost to follow-up. We have a dynamic and diverse groups of investigators that will explore the following aims, considering potential effect modification of age, sex, race/ethnicity, and HIV-status on each of these.

TABLE B.1: Specific Aims	**Interested ICO**
Cardiovascular Aims: To identify traditional, behavioral, HIV-related and novel risk factors associated with: a) incident fatal and hospitalized nonfatal CVD events; and b) development and progression of subclinical cardiac structural and functional abnormalities.	NHLBI
Pulmonary/Sleep Aims: To identify novel risk factors, biomarkers, and influence of HIV on lung function trajectory, pulmonary exacerbations, respiratory symptoms, sleep quality, and habitual sleep duration.	NHLBI
Neurocognitive Aims: To a) describe the pattern and profile of cognitive impairment and their associated risk factors and risk modifiers and b) determine alterations in MRI-measured brain structure and their association with cognitive function.	NINDS
Aging Aims: To investigate: a) longitudinal changes in physical function and factors influencing these changes; b) incidence of geriatric conditions and factors influencing these conditions, including multimorbidities; c) molecular and biochemical markers of aging; and d) the biology of healthy or successful aging.	NIA
Cancer Aims: To determine: a) the incidence of and risk factors associated with AIDS and non-AIDS related malignancies; and b) factors (e.g. psychosocial, behavioral, cancer treatment, HIV-related factors, and comorbidities) associated with cancer survivorship.	NCI
HIV Pathogenesis Aims: To evaluate factors influencing HIV persistence and the long-term latent viral reservoir in blood and tissues of a subset of well-matched HIV-infected, ART-treated men and women who have long-term plasma viral load suppression (n=80). We propose to evaluate the size of the HIV viral reservoir by sex and anatomical location and its relationship with: a) immune cell phenotypes; b) the degree of clonal expansion of latently infected CD4 T cells, and c) the microbiome and antiretroviral drug exposure.	NIAID
Psychosocial Aims: To examine: a) trajectories of vulnerabilities and resiliencies and their relationship to HIV outcomes and comorbidities; and b) mechanisms by which psychosocial/behavioral and structural predictors affect health outcomes.	NIMH, NIDA
Health Disparities Aims: To expand enrollment to men in the Southeastern U.S. that are representative of the regional epidemic in order to: a) evaluate the effect of personal (e.g., race, sex, education, income) and neighborhood-level characteristics (e.g. census tract-level poverty, county-level income inequality) on achieving prevention and treatment targets for HIV and comorbidity outcome; b) refine methods incorporating spatial information for estimating differences in achieving health guideline targets to reduce the incidence of MI and stroke, lung cancer, and all-cause and cause-specific mortality.	NIMHD
Platform Aims: To serve as a reliable, valid, and standardized platform for collaborative and independently funded research with investigators within the CCS and external to the CCS by: a) continuing collection of fresh specimens (blood, urine, oral, stool, cervicovaginal lavage, hair and tissue for tumor and reservoir studies) and measuring clinical, immunologic and virologic markers; b) expanding assessments of comorbid conditions and adjudicated clinical outcomes (e.g., CVD, ESRD, ESLD, cancer, and death); and c) developing a MACS-WIHS omics platform (e.g. genome, microbiome) and oral health protocol.	All NHGRI NIDCR
Career Development Aims: To foster the development of early career investigators and students interested in HIV research and to engage early established investigators not currently conducting HIV-related research in innovative studies leveraging the platform of the CCS.	All

Hypotheses, Significance, and Summary of Approach

Cardiovascular Aims

Hypotheses: 1) HIV is associated with a greater incidence of fatal and non-fatal CVD events; 2) HIV is associated with progression of cardiac dysfunction. Greater cumulative RNA, lower nadir and current CD4 count are associated CVD events and with progression of cardiac dysfunction with contributions from sociodemographic, lifestyle, clinical and laboratory factors, as well as genetic markers.

Significance: The association of HIV with atherosclerotic vascular disease in the coronary and carotid arteries has been well documented in the MACS and WIHS.[1-3] Recent studies have linked HIV to other forms of CVD, including heart failure[4,5] and atrial fibrillation[6]; however the mechanisms for this increased risk are incompletely understood. The longitudinal rich database from the CCS and appropriate HIV- comparison group will allow for assessment of risk for CVD events. The proposed study will be uniquely positioned to characterize the contributions of HIV and HIV-specific factors to the incidence of fatal and non-fatal CVD events and the progression of cardiac dysfunction, and allow development and study of appropriate strategies to lessen the burden of CVD in this population.

The introduction of ART has largely eliminated the fulminant dilated cardiomyopathy seen in late-stage AIDS,[7] but has given way to more insidious kinds of cardiac dysfunction in PLWH, wherein mild LV systolic dysfunction is common (~8%) and LV diastolic dysfunction is pronounced (~43%).[8] Prior echocardiographic (Echo) studies did not allow clear assessment of the role of HIV, because they were either uncontrolled or relied on healthy volunteers for their comparison group.[9] Recent NIH funding has provided the opportunity for Echo assessments in the entire MACS and WIHS cohorts. These studies will evaluate the cross-sectional associations of HIV and HIV-related factors with cardiac dysfunction in both sexes. Here, we build on this work to investigate for the first time the determinants of long-term longitudinal progression of left ventricular (LV) global longitudinal strain (GLS), left atrial (LA) strain, and right ventricular (RV) free wall strain in PLWH, leveraging speckle-tracking Echo-determined myocardial mechanics for superior detection of early-stage cardiac dysfunction.[10-12]

Approach: 1) We propose to continue to measure traditional CVD risk factors in all CCS participants and will add new measures of diet and physical activity using standardized survey instruments. We will build on existing methods of event ascertainment and develop a standardized method for medical record collection and event adjudication by a physician committee. We will adopt existing protocols used in MESA and other NHLBI cohorts. Events to be adjudicated will include hospitalized nonfatal and fatal MI, angina, heart failure, coronary revascularization, atrial fibrillation, resuscitated cardiac arrest, stroke, TIA, peripheral vascular disease revascularization, DVT and PE. 2) We will perform Echo ~5 years after the initial scans in ~3,500 participants ages 40 and older from the WIHS and MACS. Measures will include traditional echo parameters in addition to LV global longitudinal strain (GLS), LA strain, and RV free wall strain. Building on the ongoing collaboration across the WIHS- and MACS Echo Studies, we will more fully integrate the two Echo Reading Centers for the individual cohorts by creating a virtual single Lab. Echos will be repeated in each cohort using the same approaches, ultrasound machines, and ultrasound analysis systems, but images will be maintained in a single central server. Harmonization of analysis approaches will then be achieved through a combination of cross-training, assessment of inter-reader and inter-vendor reproducibility, and development of machine learning techniques for optimizing interpretation of image data across the two physical laboratories. (See Appendix)

Pulmonary/Sleep Aims

Hypotheses: 1) HIV infection and HIV-related factors (e.g. cumulative HIV RNA, nadir CD4 counts) accelerate longitudinal lung function decline, increase respiratory symptom burden, and

increase exacerbation frequency. 2) Immune activation and inflammation identify individuals at increased risk for rapid decline in lung function, more frequent COPD exacerbations, and comorbidities, mediated at least in part by abnormal inflammation. Furthermore, the inflammatory biomarkers related to lung dysfunction differ by HIV status. 3) Never-smokers, women, and PLWH have unique risk factors for pulmonary impairment.

Significance: Lung disease is a leading cause of morbidity and mortality in PLWH. HIV infection is associated with a high burden of respiratory symptoms, pulmonary function testing (PFT) abnormalities, chronic obstructive pulmonary disease (COPD), and COPD exacerbations PMID: 25942460. HIV is an independent predictor of COPD and PLWH may have accelerated decline in lung function. PLWH with COPD have a higher pneumonia risk and the presence of COPD significantly increases the risk of death in persons with HIV 29313714, 29135579. Although smoking is clearly an important risk factor for COPD, we also see significant pulmonary function abnormalities in PLWH who have never smoked and in animal models of HIV without smoke exposure. The burden of COPD in HIV will likely increase as the PLWH population continues to age. A critical knowledge gap exists in the understanding of COPD pathogenesis, progression, and treatment in HIV. These studies will build on prior cross-sectional pulmonary testing in MACS and WIHS by adding longitudinal measurements over seven years, adding evaluation of COPD exacerbations, and investigating identified sub-groups of interest. Such data do not exist in a large cohort of PLWH.

Approach: Having completed baseline PFT measures in MACS and WIHS participants during 2017-2018, we will now perform longitudinal PFTs collecting additional PFT measurements in years 2 and 5 (2020 and 2023). PFTs will be collected in 3700 participants including 3200 existing participants with PFTs in 2017-18 and 500 new enrollees. In addition, we will administer yearly follow-up questionnaires with emphasis on smoking status and pulmonary symptoms, diagnoses and medications. Every six months, we will also assess for pulmonary exacerbations with a standardized questionnaire.

- *Spirometry*: All testing will be performed by trained study personnel per ATS/ERS guidelines. Main outcome variables will be pre- and post-bronchodilator (4 puffs albuterol) forced expiratory volume in one second (FEV1) % predicted, airflow obstruction (quantified by FEV1/FVC), and lung function decline (quantified by FEV1 slope over time).
- *Diffusing capacity for carbon monoxide (DLco):* DLco will be tested in selected participants per ATS/ERS guidelines. The main outcomes will be DLco % predicted and decline over time, adjusted for hemoglobin and carboxyhemoglobin. As part of our quality assurance, all technicians will continue to participate in a skill maintenance program by submitting monthly spirometry and DLco reports for quality review. All pulmonary function testing will be reviewed at the reading center and graded for acceptability.
- *Pulmonary symptoms assessment*: Respiratory questionnaires will include the St. George's Respiratory Questionnaire (SGRQ) and the Modified Medical Research Council Dyspnea scale (MMRC). Use of respiratory medications will be collected by standardized participant interview. We will also administer a questionnaire to assess frequency of exacerbations for the prior six months at all visits. Information obtained will include number of exacerbations, exacerbation treatment, and need for hospitalization.

Sleep pilot. Sleepiness and fatigue are recognized as a major source of morbidity in those with HIV infection, and can impair quality of life and functional status. Overnight sleep monitoring will be done using the Nox A1 self-applied portable sleep monitor among 40 women (20 HIV+, 20 HIV-), to explore whether sleep quality and duration among women is poorer among HIV infected then uninfected women, and is similar in magnitude to issues observed among similar men (who had this data collected in 2018 in the MACS).

Neurocognitive Aims

Hypotheses: a) HIV status will moderate age-related comorbidities that compromise cognition via several mechanisms. b) Comorbidities and psychosocial factors will interact with HIV status to impair cognition and brain structure and function. c) Differences in patterns and predictors of CI and brain outcomes due to race, sex, and cumulative cardiovascular, psychosocial, and health disparities factors will be observed.

Significance: Sex differences in neurocognitive function and critical correlates of aging over the life course are important because they impact participants every day and the ability to cope to stressor which can impact the ability to successfully age with HIV (Rubin et al., 2017; Valdez, Rubin, & Neigh, 2016; Vance, McGuinness, Musgrove, Orel, & Fazeli, 2011; Vance, Rubin, Valcour, Waldrop-Valverde, & Maki, 2016). Knowing how the sex differences manifest within the context of aging and HIV provides insights for interventions and treatments; this also intersects with and supports the core Aging Aims. The follow-up of well-characterized participants and employing state-of-the-art structural and functional neuroimaging will allow us to map separate and common mechanisms of cognitive function, decline, and cognitive impairments (CI) while tracking changes in brain structure and activity with life course aging, cognition, and occurrences of comorbidities.

Approach: *Screening & Harmonization:* To examine "motor and neurocognitive abnormalities" as well as "behavioral and psychiatric abnormalities" as specified in the RFA, we will assess neurocognitive function among all participants at each semi-annual visit using a 10-minute, tablet-based screening battery. In addition, a longer 1 hour neurocognitive battery will be administered every 2 years (annually among those ≥65 years), using the existing MACS battery among ongoing and newly recruited men and the existing WIHS battery among existing and newly recruited women (for longitudinal consistency). A substudy of 200 female and male participants will complete both batteries and this data will be used to create a harmonized battery that will be used for participants moving from the WIHS Visit 50 and the MACS Visit 71 forward to the new CCS Visit 1 Protocol in October 2019.

Brain Structural & Functional Imaging: As an emphasis on "brain structure and function" was mandated by the RFA, MRIs are being conducted during years 1-2 on 100 women (2/3 HIV+) from 4 WIHS sites (~25 from Brooklyn, San Francisco (UCSF), D.C., and Birmingham (UAB)) as part of a cross-sectional neuroimaging study. These sites were chosen because they have resident expertise and compatible MRI capabilities (Siemens Prisma 3T software VE11b) which allow scans from each site to be examined in the same way. This data will be compared to existing structural and functional imaging (resting state connectivity, brain atrophy) currently being collected among 332 MACS participants.(Hines et al., 2016; Rubin et al., 2016; Wilson et al., 2015).

B.2.4. Aging Aims

Hypothesis: Our overarching hypothesis is that aging phenotypes and co-morbid outcomes occur earlier and with greater severity among adults living with treated HIV infection than in similar HIV- adults at risk for infection. To address this hypothesis, we will study complex characteristics of systemic aging across organ systems, particularly non-organ-specific events and changes that are associated with chronological age.

Significance: Aging in HIV+ adults is a focus of both the NIH and WHO (reference), and an area which the CCS can support with the highest quality science. The CCS is particularly well-positioned to address this because: 1) HIV+ adults have a disproportionately high number of aging-related co-morbidities; 2) the CCS data on health risk indicators(D. R. Gustafson et al., 2013) and health outcomes, and its bio-repository, collected over ≥25 years, allow us to perform focused prospective analyses defining mechanisms of health maintenance and disease progression; 3) the CCS has a publication record on HIV infection and aging phenotypes such as frailty (1-8) (D. R. Gustafson et al., 2016; D.R. Gustafson et al., 2017), grip strength (9), gait speed (10), physical performance (4), and others (falls, fractures, hearing, balance and cognition) (11-

13) (D. R. Gustafson et al., 2015; D. R. Gustafson et al., 2013; Terzian et al., 2009; Thurn & Gustafson, 2017) that span traditional organ system boundaries and reflect aging of the whole person; and 4) CCS participants are reaching a life stage during which aging-related outcomes are more common and effects of living with or at-risk for HIV will manifest most strongly (specifically, over 50% of all CCS participants are ≥50 years of age, similar to the national HIV+ population(Vance, McGuinness, Musgrove, Orel, & Fazeli, 2011)). Thus, the CCS provides a robust foundation for addressing physical, cognitive, psychosocial, and biological aging, as well as health disparities (HD) and stigma. Critical questions to be addressed are: 1) What are biochemical, molecular, genetic, cellular, and organismic processes that underlie and define aging? 2) What manifestations and measures best allow assessment of biologic *vs* chronologic aging? 3) Are these processes and manifestations accelerated in treated HIV+ adults, or is earlier occurrence due to a higher burden of comorbidities, adverse health behaviors, and HD compared to HIV- adults? Answers to these questions will have a direct bearing on global public health, health care, and policy.

Approach: In relation to each Aim we will: (a) Examine physical function (4m walk, grip strength, standing balance, and chair stands) at each CCS visit among participants aged ≥40 years, and relate these longitudinal data (including historical data) to morbidities and mortality; (b) Monitor incidence of geriatric conditions, such as falls, frailty, disability, and fractures; (c) Evaluate molecular markers of aging (e.g., epigenetic changes; miRNAs; markers of chronic diseases) using stored and newly collected biospecimens to longitudinally associate (as single measures, change and trajectories) with inflammation, body composition, disability, and geriatric syndromes; (d) Collaborate with other CCS working groups to relate longitudinal changes in physical function to changes in function of other organ systems (e.g., cardiovascular and brain aging); (e) Identify factors contributing to successful aging and survival, such as absence of co-morbidity and frailty, adequate social support, and maintenance of sexual and cognitive functions in collaboration with the Psychosocial/Behavioral Working Group.

Cancer Aims

Hypotheses: (1) Among cancer survivors, decreases in social, behavioral, mental health, cognitive and physical health domains, and access and use of care after cancer diagnosis differ by HIV status and by cancer treatment; (2) Non-AIDS-defining obesity-related cancer incidence is preceded by high BMI, weight gain, and central obesity; traditional risk factors and biomarker associations are modified by HIV infection status; (3) Lymphoma risk is reduced in early ART initiators, and levels of immune activation markers are relatively lower prior to lymphoma diagnosis. The impact of ART is not uniform and is more likely to reduce certain B-cell lymphomas but not T-cell lymphomas. The effect of ART on the grade and stage of lymphomas at diagnosis also varies. (4) The test characteristics (sensitivity, specificity, PPV, NPV) of next generation HPV biomarkers to screen for and detect anal pre-cancer, and how it differ by sex, HIV, and CD4 cell count. HPV DNA CpG methylation testing can provide risk attribution in HIV(+) women with multiple HPV type infection. HIV-related immunosuppression is a risk factor for HPV persistence and progression.

Significance: Risk of many cancers are increased in persons living with PLWH (1), and due to effective ART, the population of PLWH cancer survivors is growing (2). While cancer survivorship has been previously examined in the WIHS and the MACS (3,4), it has not been investigated in the context of social, behavioral, mental health, cognitive and physical health domains. Living with HIV infection results in easier access and more frequent use of health care. However, it is unknown if living with HIV alters access and use of cancer-related health care.
Overweight and obesity are associated with increased risk of at least 13 different types of cancer, and are responsible for 40% of all cancers diagnosed in 2014 in the U.S. (US task force report). Obesity is common (46-61%) among PLWH, with significant increases following ART initiation (5). The convergence of HIV-infection and treatment with obesity may compound the risk for

cancer in the aging PLWH population. Therefore, we will explore risk factors and potential mechanisms that link obesity, HIV, and cancer risk, which include indicators/markers of glucose–insulin homeostasis, sex hormone balance, microbial translocation, and inflammation.
Non-Hodgkin lymphoma (NHL) is an important cause of morbidity and mortality in PLWH (6-8), and even in countries with readily accessible ART still causes 23-30% of AIDS-related deaths (9-12). Elevated levels of circulating cytokines, soluble immune receptors, markers of inflammation, immune activation, and microbial translocation have been shown to precede AIDS-NHL by several years in both the MACS and WIHS (13-19). Systemic immune activation and inflammation are dampened, but not resolved, with ART (20,21). While Hodgkin lymphoma risk is also elevated in PLWH (22), its immuno-epidemiology has not previously been fully investigated in PLWH, nor has NHL following ART.

Approach: For the first hypothesis, comprehensive ascertainment of both cancer and death are a critical part of the core study protocol including cancer registry matching, national death index matching, collection of medical records, and adjudication of cancer outcomes. In addition, we will be collecting tumor tissue for prospectively diagnosed cancer cases, and a subsample of priority retrospective cancer cases in the cohort, to enable study of tumor markers that impact treatment and survival.

For the second hypothesis, a case cohort design is planned (hypotheses 2 and 3). Cases will include all participants with diagnoses of: colorectal (N=50), pancreatic (N=17), liver (N=31), kidney (N=11), myeloma (N=14), thyroid (N=14), prostate (N=84), breast (N=55), and lymphoma (n=310). Each cancer case group will be compared to a common subcohort sample without cancer, randomly selected (N=500), to assess the role of biomarkers in cancer risk. The subcohort will be randomly selected, and comparable to the case group by HIV status, cohort membership, and cART status. For each participant two archived serum specimens will be selected 1-2 years and 4-5 years prior to cancer diagnosis (or matched time in follow-up) for ~2,250 specimen visits tested for pre-defined candidate biomarkers for inflammation/immune activation (IL6, CXCL13, IP10/CXCL10, sTNFRII, TNFα), microbial translocation (sCD14, LPS-binding protein [LBP]), and metabolic/adiposite markers (insulin, IGF-1, insulin C-peptide, adiponectin, leptin). In addition, serum levels of estradiol, estrone, androstenedione, DHEAS, testosterone, SHBG will be quantified.

Lastly, we will also study HPV CpG site methylation and its relation with incident cervical precancer in HIV(+) women, and the association of microbiome with HPV persistence.

Pathogenesis Aims

Hypotheses: (1) The reservoir size and immune cell phenotypes will differ by sex and anatomical location; (2) Residual viremia occurring in certain immune phenotypes will correlate with clonal expansion, contributing to the HIV reservoir; (3) The size and composition of the reservoir will vary by microbiome composition and ART exposure.

Significance: Despite modern ART, most PLWH have residual viremia when measured by single copy assays,[1] and HIV persists in latently-infected cells which harbor the viral reservoir.[2] This HIV persistence poses a barrier to an HIV cure and long-term ART-free survival.[3] Most latently-infected cells reside in tissues, but HIV reservoir studies have focused mainly on the peripheral blood.[4] Studies suggest that important sex differences may exist in viral reservoir properties but these differences are poorly understood.[5] In fact, our preliminary data suggest that the phenotypic characteristics of the latent reservoir may differ in male- and female-specific tissues (See Atlanta and San Francisco CRS applications). Since many potential HIV cure strategies are immune-based and sex-dependent differences in immune response are well documented, understanding sex differences in cellular phenotypes that correlate with the size of the latent reservoir may optimize future cure strategies for all PLWH. Unfortunately, women are underrepresented in HIV reservoir and cure studies,[6] thereby limiting evaluation of therapies that may differentially affect the HIV reservoir in women or in female-specific tissues. HIV persistence

is likely influenced by other virologic, immunologic, microbiologic, and pharmacologic factors that also may differ by sex, such as immunologic pathways (i.e., IL-10 secretion by HIV-infected $CD4^+$ T cells[7] or IL-32 in the setting of HIV/HSV co-infection[8]), the microbiome,[9,10] and/or inadequate antiretroviral drug exposure.[11] Clonal expansion of CD4+ T cells carrying replication-competent proviral HIV may also contribute to reservoir maintenance.[12] All of these factors could potentially differ by sex.

The proposed aim is innovative in: 1) the detailed clinical and longitudinal characterization of MACS-WIHS CCS participants, allowing for the careful selection of a subset of well-matched men and women living with HIV who are maximally suppressed on modern ART, 2) the expertise of CCS investigators in cutting-edge HIV reservoir and cure research methods (such as mass cytometry [CyTOF],[13] a modified viral outgrowth assay,[14] a reporter gene-based TZA assay,[15] and integration site analysis/ proviral sequencing[16]), and 3) as one of the largest tissue studies investigating replication-competent latent HIV reservoir in men and women.

Our *overall objectives* are to (1) quantify HIV persistence and perform comprehensive phenotyping of immune cells in blood and mucosal sites (rectosigmoid and male and female reproductive tracts), and (2) determine the pharmacologic, immunologic, microbiologic, and virologic factors that contribute to HIV persistence. Infrastructure and standardized procedures developed will also provide a platform for investigators to perform HIV cure studies utilizing high quality tissue specimens from men and women living with HIV. Completion of this aim will provide critical data to inform the development and evaluation of future HIV cure strategies in men and women living with HIV.

Approach: Peripheral blood, female reproductive tract cells, seminal cells, and rectosigmoid tissue from 40 men and 40 women living with HIV receiving ART will be collected and tested (see Appendix) for the following hypotheses:

To characterize HIV persistence, we will measure: HIV-1 RNA, total proviral DNA, integrated DNA, and quantitation of replication-competent virus using both a quantitative viral outgrown assay (QVOA) combined with a novel single molecule array (Simoa)[17,18] and a TZA assay,[15] both of which offer improved sensitivity over standard QVOA and use fewer cells.[14] To understand the role of immune phenotype in reservoir size in each anatomical location, cells isolated from blood and mucosal sites from participants with the highest and lowest quartiles of reservoir size will undergo in-depth phenotyping using CyTOF. Using bioinformatics approaches, we will identify set of cellular markers that associate with reservoir size and characteristics in each anatomical location (see Appendix). As part of future studies, these markers will be analyzed using flow cytometry on specimens from other subsets and a larger sample of MACS-WIHS CCS participants to expand the findings.

For Hypothesis 2, we will utilize a single copy assay[1] to determine the presence and magnitude of residual viremia in plasma, and associate this with reservoir size in men and women. Among participants with detectable residual viremia, we will utilize single genome sequencing and integration site analysis (see Appendix) to determine whether higher residual viremia occurs due to the clonal proliferation of infected cells. We will also evaluate how T cell and innate phenotypes[19] are associated with clonal expansion.

For Hypothesis 3, we will characterize the oral, gut, and vaginal microbiome using 16sRNA and shotgun gene sequencing for metagenomics, and evaluate its relationship with the HIV reservoir. To evaluate the effect of ART exposure on the reservoir and determine whether this effect is modulated by the microbiome, we will quantify ART concentrations in small hair samples (a measure of ART exposure over weeks to months) using LC/MS/MS methods pioneered within WIHS[20] (see Appendix).

Psychosocial Aims

Hypotheses: 1) Trajectories of persistent *vulnerabilities* (e.g., mental health challenges, social isolation, stigma, substance use, and other structural/syndemic[1-3] factors) and their

interactions are associated with worse self-management of HIV, co-morbidities and health outcomes in persons living with or at risk of HIV; while *resiliencies* (e.g., coping, social support, good patient-provider relationships) are associated with better self-management and health outcomes. Further, we hypothesize that these trajectories and effects will differ between women and men; taking into account the different profiles of women and men affected by HIV. 2) The *pathways* by which these vulnerabilities and resiliencies affect self-management of HIV, co-morbidities and health outcomes can be identified. These pathways include interpersonal, mental health, psychological, and biological mechanisms[4] for effects of these vulnerabilities and resiliencies on both health behaviors (e.g., chronic disease self-management) and clinical outcomes of these diseases.

Significance: Individual, social, and environmental determinants of health and their interactions must be identified in order to identify effective, feasible, and acceptable approaches to prevent onward HIV transmission and halt HIV progression, as well as to promote overall health among men who have sex with men and racial/minority women who have been most affected by HIV.[5] Key vulnerabilities that have emerged from a rich history of psychosocial and behavioral research within the MACS and WIHS cohorts include mental health challenges[6-9] social isolation,[10] and structural/syndemic factors (low education, partner violence, incarceration and trauma,[11-13] substance use[14-19], smoking[20-23], stigma and discrimination,[24,25] food insecurity,[26,27] poverty[28], and neighborhood disadvantage[29,30]). We have also begun to elucidate important resiliencies, including social support and connectedness, positive affect, close relationships,[31,10,32] engagement in care [33,34], sexuality and sexual health,[35] and coping and resilience.[36-38] However, gaps remain in our understanding of how vulnerabilities and resiliencies affect management of HIV disease and co-morbidities, as well as directly affecting physiological systems (inflammation, immune factors, and stress-responsive systems). Resiliencies research conducted among PLWH is innovative, and can inform interventions to support remaining healthy into later life, rather than focusing on deficits.[32-34,40-42] Our proposed research agenda will identify the most salient resiliencies against poor health outcomes among PLWH and those at risk for HIV to aid in the design of these interventions. A growing body of research shows that psychosocial assets are potentially modifiable , and may therefore represent a natural leverage point for promoting health and quality of life; approaches focusing on strengths are generally more acceptable to clients and are low cost.

Approach: We will continue and expand our measurement of key behavioral, psychosocial, and structural variables in the CCS, ensuring that key variables are harmonized in a way that ensures longitudinal patterns with previously collected data are still able to be explored and are captured in the same (or appropriate complementary ways) for men and women. (See appendix for table of psychosocial and behavioral predictors and pathways that we propose to measure.) We will use statistical techniques (structural equation modeling, latent profile analysis, decision making trees) to elucidate the pathways for psychosocial, structural and behavioral predictors (both risk factors and resiliencies) on health behaviors and health outcomes, including HIV immunological and virologic outcomes, inflammatory markers, and associated co-morbidities such as cardiovascular disease, cancer, and cognitive decline. We will examine whether these mechanisms differ for women compared to men, taking into account their different social and economic contexts.

Health Disparities Aims

Hypotheses: 1) Participants who are Black, women, live in counties with high levels of economic inequality, or lack health insurance will be less likely to achieve prevention and treatment target goals for HIV and common comorbidities (achievement of smoking cessation, blood pressure <130/80 among individuals with hypertension, Hemoglobin A1C of <7.0% among people with diabetes, use of lipid-lowering agents by participants with a predicted cardiovascular risk >7.5%; performance of low-dose chest CT among participants who meet eligibility criteria for

lung cancer screening) than those who are of other race/ethnicities, men, reside in counties with less inequality, or are insured.
2) Health insurance partially mitigates the effects of poverty and income inequality on achieving prevention and treatment targets**.**

Significance: Marked, persistent inequities by race, socioeconomic status (SES), and geographic region characterize US health and mortality rates.[1,2] Chief among the complex, interacting factors that drive these inequities are poverty, the "socioeconomic context" (social and economic features of the individual's environment), and limited access to health care. [3-6] However, the specific pathways between social factors and health inequities remain unclear, a problem that thwarts development of interventions to decrease inequities. The overall goal of this research is to characterize disparities among US men and women with or at high risk for HIV infection in achievement of guideline targets and rates of health outcomes associated with demographic factors (such as age, sex, race/ethnicity, education, and income) – as well as the effects of socioeconomic context and health insurance on health outcomes. A second goal of the proposed methodological work, which emanates from and builds on a body of methods previously developed using MACS and WIHS data,[7-11] is to develop and refine innovative methods that will enable researchers to more accurately estimate the effects of potential policy interventions to reduce health inequities.[12,13]. The proposed analyses will enable us to detail how structural factors (for example, interruptions in insurance coverage) affect health outcomes – and provide evidence to guide health policy and develop effective interventions to reduce inequities**.**

Approach: At every semi-annual visit all participants routinely undergo interviews and laboratory testing that provide data concerning the proposed prevention and treatment target goals, as well as demographic and insurance information. Study staff will confirm and adjudicate disease outcomes through review of medical records and NDI matching. We will continue to geocode residential addresses of participants and merge them with census block group data to create a contextual database, which provides neighborhood-level data.

Platform Aims

Significance: The MACS and WIHS each have a long history of serving as a platform for nested research and enabling collaborations. During the previous funding cycle, there were 64 funded grants from 66 teams of scientific investigators who performed research within the MACS or WIHS studies (see letter for support about the importance of the CCS as a research platform, Appendix X).

Approach: Participants will continue to be followed every 6 months with a core visit protocol, with additional scientific subs-study protocols offered as scientifically justified (see Figure X). The core visit protocol includes: **examinations** (physical exam, oral rinse, and in women a gynecologic exam), **neurocognitive testing**, **aging and frailty assessment**, **survey** administration (behavioral, mental health, socioeconomic, screening and health care utilization, respiratory symptoms assessment), **outcome** report (self-report hospitalization and diagnoses, followed up for medical record collection), **medication** use, specimen collection (blood, urine, in women vaginal self swab), and lab testing related to HIV (In HIV+: T-cell, HIV RNA, in HIV- HIV antibody test), liver/renal function, hepatitis, metabolic panel, glucose, HgA1c, protein and creatinine. Serum, plasma, and cell samples are sent to the national repository annually on all participants.

In addition to the samples already in the repository for PBMC pellets (MACS=44,364; WIHS=411,919), lymphocytes/cells (MACS=315,392; WIHS=314,399), plasma (MACS=895,825; WIHS=713,499), and serum (MACS=721,751; WIHS=414,618), we will continue to collect these fresh specimens at each visit. The study repository is a rich source of additional sample types as well including: anal swabs (MACS=2,060), semen (MACS=58,498), cervical vaginal lavage (WIHS=53,027 pellets; 40,248 supernatants; 478,634 whole), cervical swabs (WIHS=55,871), stool (MACS=6,967) , throat washing (MACS=33,282), stimulated saliva (WIHS=13,186), urine

(MACS=89,248; WIHS=91,278 LCR; 12,439 pellets; 80,532 supernatants); B-cells (MACS=3,354), and in the NCI repository live cells (MACS=2,031), and DNA (MACS=4,034, WIHS=3,842). See the study core visit data and sample collection described in section B3.

Outcome assessment and adjudication plan: outcomes will be assessed every 6 months by interview, with self reported outcomes follow-up for medical record confirmation by study staff. In addition, outcome are collected from cancer registry, renal data system (RDS), and national death index matching, and from some of the scientific substudies (e.g. ECHO), and some outcomes are defined by existing lab testing (e.g. diabetes defined by glucose testing). The following "essential outcomes" will have centralized review and adjudication: AIDS-defining illnesses/opportunistic diseases, malignancies (other than non-melanoma skin cancers), COPD/emphysema, pneumonia, bacteremia/septicemia, ESRD, renal replacement therapy (hemo- or peritoneal dialysis, kidney transplants), bone/joint-fractures (except for fingers and/or toes only), dementia/neurocognitive disorder, diabetes (if HbA1C is not available), dyslipidemia and hypertension (only when study-obtained data are not supportive of self-reported diagnosis), and all CVD–related outcomes not elsewhere adjudicated (e.g. endocarditis). Further all prospectively self reported hospitalizations and deaths will be adjudicated, and deaths from 2005 to 2017 for which records can be obtained will be adjudicated. Adjudication will be performed by a working group of clinician investigators with expertise in xxx, << will briefly describe suggested process here >> Adjudication review and coding will be facilitated by the DACC which will facilitate and track review.

Quality control procedures: Multiple quality assurance steps are taken to ensure the highest quality of study data. Quality control includes: standardized visit protocols with regular coordination calls across sites, site visits by the DACC to evaluate adherence to and implementation of current protocols, evaluation of study data for completeness and accuracy, rigorous editing procedure including checking for ID issues (missing, duplicated, invalid), checks against dates, range checks, internal consistency checks and cross-form check. IN addition, after each visit, 5 participants per site are selected by the DAAC and raw forms are submitted and compared to received electronic data.

Career Development Aims

Significance: The CCS scientific agenda provides unparalleled opportunities for early stage investigators and investigators who are new to HIV to engage in multidisciplinary HIV research across the translational spectrum. Since their inception, both the MACS and the WIHS have served as invaluable platforms for career development of investigators at all stages. There are currently 13 NIH-funded mentored career awards, as well as several R awards led by established investigators who leveraged the MACS and/or WIHS platforms to expand their research programs into the HIV population including Drs. Wendy Post, a cardiologist, Robert Kaplan, a cardiovascular epidemiologist, and Michael Shlipak, a kidney epidemiologist. Furthermore, MACS and WIHS investigators are committed mentors and teachers, many of whom direct research training programs across multiple disciplines including T32 and K12 training programs; 5 longstanding MACS and WIHS investigators have K24 mentoring awards.

We therefore propose to build upon the strengths in career development of the CCS by each CRS site allocating funds to support career development and establishing an Investigator Developmental Award Advisory Committee (IDAC) that will serve as an advisory group with the goal of fostering career development within the goals of the MWCCS scientific agenda.

Approach: (1) To establish a funding mechanism to support the career development of CCS investigators. (2) To create the IDAC to support the administration of career development funds and foster the careers of early stage investigators and investigators new to HIV research.