SPECIFIC AIMS: More than 1 million people in the US are HIV infected (HIV+).1 With the success of anti-retroviral therapy (ART) and improving long-term survival, diseases of aging, such as cardiovascular disease (CVD), are now important health problems facing HIV+ people.2-3 HIV infection is a CVD risk factor.2-7 Among HIV+ people, ART, Framingham risk factors, anemia, hepatitis C co-infection, and renal disease are CVD risk factors.8-10 However, these risk factors do not completely explain the excess risk of CVD among HIV+ people.

CVD remains the leading cause of death in the United States11 and a major cause of morbidity and mortality world-wide.12 The underlying cause is largely a function of progressive atherosclerosis, which in turn is caused by inappropriate lipid metabolism, and activation of the innate and adaptive immune systems in the arterial wall.13-15 However, the evidence supporting these latter mechanisms involving innate and adaptive immune cell types (e.g., monocytes and T cells) and subsets (e.g., TH1 cells) and CVD is largely confined to murine models.16-20 Alteration in immune cell function is a shared feature of CVD and HIV pathogenesis.14, 21 Whether the activation, number and or proportion of peripheral circulating monocyte and T cell subsets are associated with incident CVD in humans and explain the excess risk of CVD among HIV+ people is not known.

Of the few human studies linking type of peripheral blood immune cell subsets (e.g., TH1 CD4+ T cells) to an increased risk of CVD events22-25 and subclinical atherosclerosis,26-35 none comprehensively examined multiple types of innate and adaptive immune cell subsets. Moreover, none of these studies examined T-helper regulatory (T\(_r\),reg) cells, potentially an important anti-atherosclerotic force, or immunosenescence (e.g., low ratio of naïve: memory T cell) and the risk of incident CVD events among HIV+ and uninfected (HIV-) people.

Our overall hypothesis is that alterations in immune cell function are associated with incident CVD and will explain the excess risk of CVD among HIV+ people compared to HIV- people. More specifically, we hypothesize that people with a higher proportion proatherosclerotic (e.g., intermediate monocytes, T\(_r\),1), a lower proportion or depletion of anti-atherosclerotic (e.g., T\(_r\), regulatory) immune cells, respectively, and/or (3) increased evidence of immunosenescence (i.e., a low ratio of naïve: memory T cells) will have increased CVD risk and that these types of immune cell subsets will mediate the main effects of HIV on incident CVD events.

To test these hypotheses, we will leverage the existing Veterans Aging Cohort Study biomarker cohort (VACS BC), a longitudinal, prospective observational cohort of 2,378 (1525 HIV+ and 853 HIV-) Veterans. All participants have stored cryopreserved cells and existing data on biomarkers of inflammation (interleukin-6, IL-6), coagulation (D-dimer), and monocyte activation (soluble CD14). Using the existing infrastructure from R01HL095136 (PIs, Freiberg and Justice, Co-I Tracy), Drs. Matthew Freiberg and Russell Tracy (Co-PIs on this proposal) will link these biospecimens and biomarker data to (1) longitudinal survey data, (2) VA electronic medical record (EMR) data, including all pharmacy, laboratory, radiology, pathology, clinical measurements, and clinical notes; (3) Medicare, Medicaid, VA fee for service, mortality, and national death index data; and (4) adjudicated acute myocardial infarction (AMI) events within and outside the VA health care system.

We propose to collect the following new data: (1) measurements of immune cell types and subsets using previously collected cryopreserved cells from 2005-2006 on all 2,378 VACS BC participants and (2) adjudicated CVD events (i.e., AMI, coronary heart disease, ischemic stroke, and heart failure and CVD death) from 2005-2017 using established protocols. By linking these new data with existing VACS data, Drs. Freiberg, Tracy, and Amy Justice (Co-I on this proposal and PI of the VACS) will complete the following specific aims.

**Aim 1:** To determine the number and proportion of pro and anti-atherosclerotic immune cells as well as naïve and memory/effector T cells among HIV+ and HIV- Veterans

**Hypothesis:** HIV+ Veterans will have lower numbers of T cells but a higher proportion of proatherosclerotic and a lower proportion of anti-atherosclerotic immune cells compared to HIV- Veterans.

**Hypothesis:** Immunosenescence will be more common among HIV+ compared to HIV- Veterans

**Aim 2:** To determine the independent association between pro and anti-atherosclerotic immune cells, immunosenescence, and prevalent CVD and incident CVD

**Hypothesis:** Veterans with a higher proportion of proatherosclerotic and a lower proportion of anti-atherosclerotic immune cells will have a greater prevalence of CVD and higher risk of incident CVD.

**Hypothesis:** Immunosenescence will be associated with a higher CVD prevalence and risk of incident CVD.

**Aim 3:** To determine if the effects of HIV infection on incident CVD are mediated by the proportion of pro and anti-atherosclerotic immune cells and immunosenescence

**Hypothesis:** The association between HIV and CVD events will be mediated by a higher proportion of proatherosclerotic and a lower proportion of anti-atherosclerotic immune cells, and immunosenescence.

If our hypotheses are true, we will advance our understanding of how immune function contributes to CVD for HIV+ and HIV- people while also potentially identifying new targets for future CVD intervention studies.