SPECIFIC AIMS: More than 1 million people in the US are HIV infected (HIV+). With the success of anti-retroviral therapy (ART) and improving long-term survival, diseases of aging, including cardiovascular disease (CVD), are now critical health problems facing HIV+ people. HIV+ people have a 50% higher risk of acute myocardial infarction (AMI) compared to uninfected people. Among HIV+ people, traditional Framingham risk factors as well as ART, anemia, hepatitis C co-infection, metabolic abnormalities, and renal disease are all CVD risk factors. However, these risk factors do not fully explain the excess risk of CVD among HIV+ people nor, when incorporated into risk prediction models, acceptably predict CVD risk in this population.

Current approaches designed to elucidate the underlying mechanism for the excess risk of CVD and mortality among HIV+ people while also identifying those HIV+ people at greatest risk for CVD are limited in two major ways: 1) the majority of prior studies focused on a small number or even a single specific biomarker to explain what is likely a very complicated mechanism and 2) such studies typically only assessed whether the incorporation of one specific biomarker would substantially improve CVD risk prediction. While prior research examining a single protein biomarker (e.g., hsCRP) has provided some insights into the association between inflammation and CVD, hsCRP alone does not fully explain the association between inflammation and CVD nor does its inclusion substantially improve existing CVD risk prediction models. We hypothesize that large-scale proteomics will identify important new CVD biomarkers/mediators and biological pathways in HIV. Aptamer proteomics (SOMAscan, SomaLogic, Boulder CO) allows for rapid quantification of over 1100 proteins in a small volume of blood, making this proteomic technology ideal for large cohort studies. We have already utilized SOMAscan in the Heart and Soul and HUNT3 cohorts of over 2000 subjects with stable coronary heart disease to identify 200 protein biomarkers prognostic of cardiovascular and mortality events (Preliminary Data). We have applied bioinformatic approaches to these proteins to derive and externally validate a 9-protein cardiovascular risk model which is superior to risk models derived from traditional risk factors. In this application, we hypothesize that by using large-scale proteomics, we can identify novel protein biomarkers that (1) will be associated with incident CVD and mortality events in HIV, (2) will generate new information about biological pathways unique to HIV-associated CVD, and (3) can be used to improve CVD risk prediction in HIV+ people. Importantly, results from this study may identify novel proteins that could potentially serve as new targets for pharmacologic therapies to treat CVD in HIV.

We will leverage the existing Veterans Aging Cohort Study Biomarker Cohort (VACS BC), a longitudinal, prospective observational cohort of 1525 HIV+ and 853 uninfected veterans. All participants have stored plasma, existing data on biomarkers of inflammation, measures of immune function (T cell, B cell, and monocyte subsets), and adjudicated CVD outcomes from 2005 to 2020. Using the existing infrastructure from R01HL095136 (PIs, Freiberg and Justice), Drs. Hsue, Ganz, and Freiberg (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 CVD events within and outside the VA health care system.

We propose to collect the following new data: measurements of 1130 plasma proteins using SOMAscan technology on previously collected plasma specimens from 2005-2006 on all 2,378 VACS BC participants. By linking these new data with existing VACS data, we will complete the following aims:

**Aim 1:** To discover a broad range of protein biomarkers predictive of CVD and mortality events in HIV.

1a) To discover novel proteins associated with CVD/mortality events, not recognized by candidate approaches in HIV using a large scale proteomics approach. **Hypothesis:** Proteins associated with incident CVD events/mortality will differ between HIV+ and uninfected veterans.

1b) To elucidate biological pathways of CVD in HIV by applying pathway analysis to the prognostic proteins discovered in Aim 1a. **Hypothesis:** Pathway analyses, focused on atherosclerotic and thrombotic pathways, will allow us to identify proteins that have a plausible biological relationship with CVD and death outcomes in HIV.

**Aim 2:** To identify a small (parsimonious) multi-protein panel of biomarkers among HIV+ people that predicts CVD outcomes and mortality events. **Hypothesis:** A multi-protein cardiovascular risk model will be identified that delivers excellent performance in HIV.

**Aim 3:** To evaluate the relative prognostic utility of a proteomics risk prediction model compared to traditional, Framingham based clinical risk models in subjects with HIV. **Hypothesis:** Risk assessment based on the multi-protein panel identified in Aim 2 will be superior to traditional risk factors and common laboratory tests for predicting CVD and mortality events in HIV-infected individuals.

If we are successful, we will identify new protein biomarkers/mediators of CVD and mortality in HIV+ people while also deriving new biological insights into the excess risk of CVD in HIV.