MIneralocorticoid **R**eceptor **A**ntagonism for **C**ardiovascu**L**ar h**E**alth in **HIV**
The MIRACLE HIV Study

I. Background and Significance

Cardiovascular related complications are a leading cause of morbidity and mortality in well-treated HIV patients in whom we have shown increased activation of the renin-angiotensin–aldosterone system (RAAS). The availability of newer antiretroviral therapies (ART) with improved efficacy, safety and tolerance has allowed HIV patients to achieve good virologic16 control and reduce the burden of AIDS defining illnesses. In this regard, HIV patients are living longer and the focus of their healthcare is now driven by chronic co-morbidities. Among these co-morbidities, large cohort studies of the well-treated HIV population have revealed that cardiovascular disease (CVD) accounts for approximately 10% of all deaths[1,2] and CVD is 1.5-2 fold higher in the HIV population compared to a matched non-HIV population.[3,4] This proposal will provide evidence supporting mineralocorticoid receptor (MR) blockade as a novel strategy for CVD risk reduction in HIV. We present significant data demonstrating for the first time impaired physiological regulation of RAAS leading to excess RAAS activation among HIV patients. We demonstrate significant increases in aldosterone linked to metabolic abnormalities, including visceral fat accumulation, insulin resistance and inflammation in HIV and further hypothesize that RAAS activation has critical effects on CVD in HIV.

Unique RAAS physiology may modulate increased burden of CVD in HIV. The RAAS is an important hormone system, which classically regulates blood volume and sodium balance through MR activation in epithelial tissues. Compelling data from animal and human studies suggest that RAAS activity may also contribute to the development of vascular and myocardial injury, largely through stimulatory effects on inflammation and fibrosis. These data and new observations by our group on the effect of blocking RAAS activation suggest a compelling management strategy aimed at reducing CV related morbidity and mortality in this at-risk HIV population, for whom comprehensive strategies to reduce CVD are lacking.

HIV patients have increased risk of vascular inflammation and subclinical atherosclerosis, which may be addressed by MR blockade. Using aortic FDG-PET, we have shown direct evidence of increased arterial inflammation among HIV patients.[5] While studies show that HIV patients have impaired endothelial dependent vasodilation vs. non-HIV patients,[6] functional assessment of this inflamed coronary vasculature in HIV is lacking. Moreover, ART treated HIV patients have persistent elevations in ICAM and VCAM,[7,8] markers of vascular inflammation which correlate with the extent of atherosclerosis in the non-HIV population.[9] Using coronary CTA, we have shown increased subclinical atherosclerosis in HIV and demonstrated the plaque most often to be noncalcified with high risk features, including positive remodeling and low attenuation—plaque characteristics more vulnerable to rupture.[10] Indeed, noncalcified plaque is distinct from long-standing, hardened calcified plaque, which is more often associated with traditional CV risk factors than noncalcified plaque. In this regard, we have established a significant link between noncalcified plaque and specific markers of inflammation and immune activation, soluble CD163 and CD14 and Lp-PLA_2, in HIV as opposed to traditional risk factors.[11-13] Moreover, progression of carotid intima-media thickness over 24 months was associated with increased baseline urinary aldosterone excretion (preliminary data r=0.67, P=0.049) in HIV. Taken together, these data suggest inflamed coronary vasculature and high risk plaque in HIV, in whom our preliminary data suggest a potential linkage of inflammation and atherosclerosis to RAAS activation. In this proposal, we will test the hypothesis that Mblockade addresses these unique

features of CVD in HIV.

Coronary flow reserve (CFR) is a physiologic marker of vascular function of the coronary bed, which is reduced in HIV. CFR, the ratio of maximally stimulated to rest coronary blood flow (CBF), is well-validated to characterize coronary vasculature function.[14-16] Both large vessel coronary lesions (i.e. coronary artery disease [CAD]) and microvascular dysfunction may lead to impaired CFR. Our group and others demonstrated that impaired CFR predicts cardiovascular morbidity and mortality in non-HIV populations. Specifically, we found impaired CFR (median 1.9) to be associated with a 4.9-fold increase in CV mortality among non-HIV patients.[17] Furthermore, CV mortality is increased among non-HIV patients with impaired vs. normal flow reserve (4.2 vs. 0.4%/yr).[17] In addition, 59% of patients with non-obstructive CAD have a reduced CFR <1.93 on cardiac PET,[15] and impaired CFR predicts cardiac mortality independent of obstructive disease.[16,18] While CFR may be a useful physiologic biomarker for CVD, few studies have examined CFR in HIV. A short 5 week study reported reduced CFR in HIV patients after initiation of ART.[19] In this proposal, we present new preliminary data demonstrating greater impairment of CFR in HIV compared to non-HIV subjects[20](1.74±0.73 vs. 3.88±0.70) assessed by cardiac PET, the gold standard for evaluation of CFR. These data highlight CFR as a diagnostic tool to uniquely capture those asymptomatic patients of intermediate CVD risk with potential limitations to flow reserve of the coronary microvasculature, which may contribute to ischemia, a clinical scenario relevant to HIV patients with increased subclinical plaque progression. Importantly, we show that MR blockade improves CFR, suggesting it may be a useful strategy to improve vascular dysfunction in HIV. Reduced stress myocardial blood flow on cardiac MRI may be another marker of reduced myocardial perfusion and has been independently associated with major adverse cardiovascular events among those without known CVD.

In addition to reduced CFR, HIV patients demonstrate myocardial fibrosis and diastolic dysfunction which may also uniquely contribute to CVD in HIV. Myocardial fibrosis is characterized by expansion of the extracellular matrix and accumulation of interstitial collagen marked by increases in circulating PINP and PIIINP.[21,22] Cardiac MRI with T1 mapping is a sophisticated technique we have validated to quantify myocardial extracellular volume (ECV) and is a standard method for non-invasive assessment of myocardial inflammation and fibrosis in lieu of an endomyocardial biopsy. Data suggests a relative increase in ECV by 10% is linked to a 50% increase in adverse CV events.[23] Myocardial fibrosis contributes to impaired diastolic relaxation and is a key mediator of diastolic dysfunction. Myocardial damage is linked to a 4-fold increase in heart failure risk.[24] Importantly, the progression to heart failure is preventable, as pathological remodeling in the myocardium related to these fibrotic changes is reversible[25-27] prior to establishment of diastolic dysfunction. In a cross-sectional study, Holloway et al. demonstrated that a majority (76%) of ART treated HIV patients had evidence of myocardial fibrosis on cardiac MRI compared to only 14% of well-matched non-HIV patients.[28] Furthermore, a meta-analysis reported that diastolic dysfunction is prevalent in approximately 43% of HIV patients.[29] Despite the alarming prevalence of myocardial fibrosis[30] and diastolic dysfunction in HIV, no longitudinal studies have been performed to date assessing changes in myocardial structure in HIV over time, nor have there been any interventional studies of strategies to regress changes in cardiac remodeling to prevent CV events in HIV.

Mechanistic pathways leading to impaired cardiovascular structure and function in HIV are not well understood. The conventional paradigm for CVD presumes a higher prevalence of traditional risk factors. Indeed, dyslipidemia, hypertension, and diabetes are all more prevalent in HIV vs. non-HIV patients, and smoking rates are increased almost 2-fold among HIV patients, but these traditional risk factors only account for 25% of the increased risk of CVD in the HIV compared to the non-HIV population.[3,4] In addition, emerging studies have provided insight on the benefit of ART use on CVD, such that viral suppression may be associated with a reduction in CV events.[31] While immunologic control is important for CVD risk reduction, ART alone only partially mitigates this risk. Therefore, our knowledge of CVD in HIV is incomplete, and significant advances in this area are needed to further our understanding of specific HIV-related mechanisms that may account for the excess burden of CVD. Mechanisms implicated in cardiac injury and dysfunction specific to controlled HIV infection may include persistent inflammation and immune activation or abnormal fat redistribution. In this grant, building on data from our existing grant, we hypothesize that increased RAAS activation in HIV may be a mediator of structural and function changes of the heart either directly through vascular injury or indirectly through stimulating a pro-inflammatory, pro-fibrotic milieu.

Evidence from animal studies and studies of non-HIV patients demonstrate that MR activation is linked to vascular dysfunction, vascular inflammation, and subclinical atherosclerosis. We and others have shown that MR activation leads to coronary vascular inflammation and dysfunction.[32] Aldosterone mediated vascular injury is associated with an increase in inflammatory and vascular markers, such as MCP-1, IL-6, ICAM and PAI-1.[33-35] In animal models of aldosterone mediated injury, such as rodents infused with Ang II and a NO synthase inhibitor[36] and double transgenic rats for the human renin and angiotensinogen genes,[37] blocking the actions of aldosterone with an MR antagonist or removing the aldosterone source through adrenalectomy reduces vascular damage and myocardial inflammation. These beneficial effects of MR blockade are independent of changes in blood pressure (BP).[38] We have shown that diabetics without known CAD have impaired CFR and subsequently, treatment with a MR antagonist (eplerenone or spironolactone) improves coronary vascular function marked by improvements in CFR.[39,40] Moreover, aldosterone may have direct effects on atherogenesis through increased oxidative stress and foam cell formation.[41] Under condition of RAAS activation, development of atherosclerotic plaque increased 3-fold in apolipoprotein E-deficient mice.[42] Moreover, MR blockade attenuated the pro-atherogenic effects of RAAS activation[43] by reducing oxidation, cholesterol accumulation, and inflammation. Other animal studies have similarly shown that eplerenone can inhibit atherosclerosis[41] independent of BP lowering effects.[44] Data in mice also show that aldosterone promotes T cells and monocyte infiltration into plaque prone regions and is associated with an unstable lipid laden and inflammatory plaque phenotype prone to rupture.[45] Moreover, eplerenone reduces expression of IL-6 and MCP-1 in macrophages during early atherosclerosis development in mice.[46] While a few human studies in non-HIV patients have been performed to support these preclinical findings linking aldosterone to plaque progression,[47] no studies have evaluated MR activation and blockade on CVD indices in HIV, a population in whom RAAS activation may indeed contribute to abnormal coronary vasculature and progression of plaque.

MR activation is also related to myocardial fibrosis and diastolic dysfunction. Animal studies have demonstrated that MR activation by aldosterone or

deoxycorticosterone acetate contributes to cardiac fibrosis and adverse remodeling of the coronary vasculature, independent of BP changes[48] Moreover, preclinical data show that Ang II-stimulated changes in myocardial inflammation, myocardial necrosis and PAI-1 expression in coronary endothelial cells were attenuated with MR blockade.[36,38] Myocardial fibrosis secondary to tissue injury by aldosterone can increase LV hypertrophy, reduce vascular compliance, and impair diastolic dysfunction. Indeed, MR blockade with spironolactone or eplerenone markedly improves CV morbidity and mortality related to heart failure in human studies,[49-51] and may have more efficacy in the HIV subpopulation in whom we have demonstrated significant RAAS activation. We have shown that 24 hour urinary aldosterone excretion is correlated with ECV in diabetics.[52] Studies in non-HIV patients complement these findings, demonstrating that MR blockade improves diastolic dysfunction,[49,53,54] and is also linked to improved markers of collagen turnover and myocardial fibrosis in those with the metabolic syndrome.[53] Similar results were confirmed among non-HIV patients, in whom eplerenone treatment improved measures of diastolic dysfunction and reduced markers of collagen turnover, including P1NP and PIIINP.[55,56] Lipocalin 2 has been implicated in mediating the profibrotic and tissue remodeling activity of mineralocorticoids through its action in cardiac myocytes, endothelium, and vascular smooth muscle cells.[57] In human studies, lipocalin 2 is associated with adverse CV events[58] and correlates with aldosterone levels.[59] Taken together, this evidence suggests that MR blockade may have significant benefit in HIV, a population with a high prevalence of myocardial fibrosis and diastolic dysfunction.

Increased RAAS activation contributes to inflammation and metabolic complications in HIV, suggesting the utility of MR blockade for CVD in HIV. Together the Co-PI's Grinspoon and Adler conducted the first comprehensive investigation of the RAAS in HIV. Initial data revealed increased urinary aldosterone in HIV vs. controls in association with increased visceral adipose tissue (VAT) and HbA1c.[60] New published data from the Co-PIs further suggests that HIV subjects with relative increases in abdominal fat accumulation demonstrate significantly increased RAAS activation under standardized dietary and posture conditions. Also, increases in aldosterone are associated with insulin resistance.[61] Furthermore, markers of inflammation, including hsCRP and IL-6, are increased under conditions of RAAS activation among HIV patients. Indeed, these novel studies performed in HIV serve as a paradigm to suggest a previously unexplored mechanism through RAAS activation by which HIV patients with an adverse metabolic phenotype are predisposed to CVD. Indeed, increased VAT is a predictor of subclinical CVD in HIV[62] and, this proposal will test the hypothesis that increases in RAAS activation contributes to CVD in HIV. We will obtain novel information on the link between RAAS activation and myocardial and vascular injury in HIV through these studies. To that end, other studies have indicated that aldosterone levels independently correlate with CV mortality.[63,64] Importantly, we will assess for the first time in HIV the specific contribution of MR signaling to multiple cardiac endpoints, including impaired CFR, myocardial fibrosis, diastolic dysfunction, and subclinical atherosclerosis, by applying MR blockade in an interventional trial among HIV patients selected based on the presence of metabolic abnormalities associated with RAAS activation and related subclinical atherosclerosis.

Summary and Implications: MR signaling may have an adverse role in CVD through vascular dysfunction, abnormal collagen deposition, vascular and myocardial inflammation, and increased oxidative stress[65]. The burden of structural and functional

heart disease in HIV is greater compared to the general population. We have demonstrated significant RAAS activation in HIV patients with metabolic abnormalities, including increased visceral adiposity and insulin resistance, and suggest that this unique physiology may contribute to a metabolic phenotype that is pro-inflammatory, pro-fibrotic, and pro-atherogenic. It is plausible that a cascade of excess MR activation in HIV potentiates cardiac injury and dysfunction. Moreover, there is relevant data to support testing the efficacy of MR blockade in managing impaired myocardial perfusion, myocardial fibrosis, diastolic dysfunction, and plaque. The grant will provide new mechanistic insight and enhance our understanding of CVD in HIV. We will take advantage of advanced imaging methods to permit a non-invasive longitudinal assessment of structural and functional CV defects in HIV to add new information to the field. This is an ideal proposal aiming to integrate several manifestations of CVD in HIV through a novel hormone pathway. The clinical utility of MR blockade could have far reaching effects towards overall CV risk reduction in HIV, and such studies could serve as a model for other at-risk metabolic groups.

II. Specific Aims

We will perform a randomized double-blind study among HIV subjects with increased visceral adiposity and subclinical atherosclerosis, administering eplerenone vs. placebo for 12 months. We will provide all subjects with lifestyle modification throughout the study. We hypothesize that adding eplerenone to lifestyle modification will significantly improve key indices of cardiac structure and function.

Among the large population of HIV patients in whom we have shown renin-angiotensin-aldosterone activation and subclinical atherosclerosis, we will determine:

- **Specific Aim I: The effect of MR blockade on myocardial perfusion.**
  We hypothesize:

a) MR blockade will improve coronary flow reserve (CFR) on cardiac positron emission tomography (PET) or stress myocardial blood flow (sMBF) on cardiac magnetic resonance imaging (MRI).

b) MR blockade will reduce markers of vascular dysfunction.

- **Specific Aim II: The effect of MR blockade on myocardial inflammation and fibrosis.** We hypothesize:

a) MR blockade will reduce myocardial extracellular volume fraction, a measure of myocardial fibrosis and inflammation, assessed by serial myocardial T1 mapping cardiac magnetic resonance imaging (MRI).
b) MR blockade will improve markers of inflammation and immune activation.
c) Reduced myocardial fibrosis and inflammation resulting from MR blockade will relate to improvements in diastolic dysfunction evaluated through diastolic strain by tagged myocardial imaging on cardiac MRI.

- **Specific Aim III: The effect of MR blockade on coronary plaque**.
  We hypothesize:

a) MR blockade will result in plaque regression and reduced high risk plaque Morphology on coronary computed tomography angiography (CTA).
b) Baseline CFR will relate to coronary plaque features in HIV patients with subclinical atherosclerosis.
c) Improvements in coronary plaque with MR blockade will relate to improvements in CFR and inflammation.

Optional Substudy--

- **<u>Specific Aim IV:</u> The effect of RAAS activation on systemic inflammation and specific indices of arterial inflammation.**

We hypothesize:

a) Markers of systemic and arterial inflammation will be increased during the RAAS activated state carefully controlled conditions of sodium restriction) when compared to the RAAS suppressed state (under carefully controlled conditions of sodium loading).

b) Higher serum aldosterone levels during the RAAS activated state (under carefully controlled conditions of sodium restriction) will be related to greater in situ arterial inflammation measured by aortic TBR using cardiac 2-deoxy-2-18F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography (^{18}F-FDG-PET/CT).

- **<u>Specific Aim V: The effect of MR blockade on arterial inflammation.</u>**

We hypothesize:

a) Treatment with MR blockade for 12 months will reduce in situ arterial inflammation measured by aortic TBR using cardiac^{18}F-FDG-PET/CT.

b) Treatment with MR will reduce markers of systemic and vascular inflammation.

c) Changes in aortic TBR will be related to changes in markers of arterial inflammation.

Optional Study for those who do not qualify following both screen visits—

- **<u>Specific Aim VI: The degree of inflammation and cardiometabolic disease among those with/without visceral fat and those with/without coronary plaque</u>**

We hypothesize:

a) Worsening markers of inflammation and heart disease across the spectrum stratified by presence and absence of excess VAT and presence and absence of coronary plaque (i.e increased worsening across the following groups: low VAT/absence of plaque, low VAT/presence of plaque, high VAT/absence of plaque, high VAT/presence of plaque).

III. Subject Selection

Inclusion/Exclusion:

<u>Inclusion Criteria</u>: Up to 160 HIV male and female subjects will be consented to achieve the number of evaluable subjects. 42 subjects will be consented for the optional study.

Subjects will be included for:

1) Ages 40-65 years
2) Antiretroviral use (ART) >12 months and viral load <100 copies/mL
3) VAT> 110cm^2

<u>Exclusion Criteria</u>:

1) Antihypertensive use including, ACE Inhibitor, ARB, MR blockade, diuretic, potassium (K) supplementation; or BP>140/90 mmHg. Stable use (>3 months) of beta-blockers or calcium channel blockers (CCB) (except verapamil) is allowed.
2) Unstable statin use <12 months. Stable use (>12 months) is allowed.
3) Use of full dose ritonavir, nelfinavir, clarithromycin, and other strong inhibitors of CYP3A4, as well as CYP3A4 inducers.
4) Continuous oral steroid use (equivalent to prednisone <u>≥</u> 5 mg daily) within the last 3 months.

5) Uncontrolled diabetes requiring insulin and/or HbA1c > 7.5%.
6) Creatinine (Cr) > 1.5 mg/dL or estimated GFR<60 mL/min/1.73m^2.
7) K > 5.5 mEq/L.
8) Hemoglobin < 10 g/dL.
9) Known liver disease or ALT >3x ULN.
10) History of congestive heart failure, stroke, myocardial infarction, or known coronary artery disease.
11) Pregnant, actively seeking pregnancy or breastfeeding. Women of childbearing age may be on a non-hormonal form of contraception.
12) Estrogen, progestin derivative, or other sex steroid use within last 3 months. Stable physiologic testosterone replacement (> 3 months) is acceptable.
13) Current bacterial or other infections.
14) Active substance abuse.
15) Significant radiation exposure over the course of the year prior to randomization (e.g., radiation therapy, PCI, catheter ablation of arrhythmia) within 12 months of randomization.
16) Previous reaction or contraindication to gadolinium.
17) Coronary artery luminal narrowing >70% on coronary CTA.

Source of subjects and recruitment methods:
Participants will be recruited from community flyers and announcements at HIV focused community centers, community newspaper advertising, community on-line websites, Partner's- affiliated Recruitment Tools, and referrals from infectious disease physicians and primary care physicians familiar with the study and through the utilization of the Partners Research Data Registry (RPDR). Potential participants, from all referral sources, will contact the study investigator directly by phone for a pre-study evaluation via telephone to determine eligibility. In addition, if a provider obtains a subject's permission for us to contact them directly, then we may do so. We will not contact any subjects for whom permission was not obtained in advance by their medical providers. For RPDR, we will perform an RPDR search to identify patients with HIV infection, ages 40-65 without known diagnosis of Cardiovascular Disease. We will subsequently review the resulted medical records to hone in on those that match our inclusion/ exclusion criteria. We will directly contact patients who have agreed to be approached by research staff as part of the Research Opportunities Direct to You (RODY) Program. For patients not enrolled in RODY, we will contact each eligible patient's provider to request that s/he mention the study to the patient and ask that individual for permission to be contacted by the study staff. Only if permission is granted, we will call the patient to conduct a phone screen and provide study information.

IV. Subject Enrollment
Informed consent will be obtained at the screening visit by a licensed physician or nurse practitioner, prior to any procedures or interventions. Potential research subjects will have the entire protocol explained in detail and study investigators will review the consent form item by item, answering any and all questions. Once risks and benefits have been reviewed and subjects have agreed to participate by signing the consent, the screening visit will continue as described in the subject selection section. Only patients capable of providing informed consent will be enrolled. Once final eligibility has been determined subjects will be scheduled for a baseline visit.

Optional Study Participation:

Upon confirmation of eligibility, subjects will be given the option through a formal consenting process to participate in the substudy prior to randomization. Participation in the main study will not be affected if subjects choose to decline participation in the substudy; however, subjects withdrawn from the main study will be withdrawn from the substudy.

If subjects do not qualify for the main study following completion of both screening visits, subjects will be given the option to participate in an optional blood sampling visit.

Treatment assignment and randomization:
During the Baseline Visit randomization will be done. Subjects will be randomized 1:1 to receive eplerenone PO 50mg daily or identical placebo for 12 months. The randomization list, stratified by gender, statin use, and presence of plaque will be generated by the biostatistician and kept by the research pharmacy. Daily dosing starts at 50 mg for the first week to avoid hypotension and will be increased to 50 mg twice daily at the 1 week safety visit. The study will be double-blinded. All subjects will receive lifestyle modification as described below.

Prior to randomization in all cases, the subject's primary care physician will be consulted regarding the appropriateness of the protocol including the lifestyle modification program with the subject's permission. Subjects are not eligible if they do not meet all of the enrollment criteria.

In the event that a subject wishes to withdraw from the study, the subject will be asked to return all unused study medication.

V. Study Procedures

Data Collection

Screening Visit 1 (to determine eligibility)**:**
We will obtain:
1) Informed consent, history and physical, urine pregnancy test for females.
2) Blood for Cr, K, ALT, CBC, HbA1c, and HIV viral load.

Screening Visit 2 (to determine eligibility)**:** Subjects will present after a 12 hr fast. If eligible from Screening Visit 1, we will obtain:
1) Urine pregnancy test for females as appropriate.
2) Coronary CTA for coronary plaque assessment. (This will not be performed if the subject has a previous reaction or contraindication to iodine-containing contrast media).
3) CT Abdomen for single slice screening at L4 for VAT determination.

Optional Blood Sampling Visit (For those who do not qualify after both screening visits): Subjects will present after an overnight 12 hr fast.
1) Fasting blood for markers of heart disease and inflammation

Bionutrition Visit (prior to both Baseline Visit 1 and 12 Month Visit): Subjects will be tested on an ad lib diet, after an overnight 12 hr fast. We will obtain:
1) Blood for Cr, K, lipids, glucose, insulin, CD4/8, HIV viral load (12 Month Visit only) and biomarkers for vascular dysfunction (sICAM, sVCAM), inflammation/ immune activation (CRP, IL6, MCP-1, sCD14, sCD163), subclinical injury (BNP, high

sensitivity cardiac troponin T [hs-cTnT]), and fibrosis (galectin-3, cardiotrophin 1[CT1], lipocalin 2, PINP, PIIINP). DNA collection for genetic testing is optional.
2) 24 hr urine collection for sodium (Na) and Cr for sodium balance will be started one day prior to the visit. In consultation with the bionutritionist, broth packets will be given to achieve standardized sodium conditions (urinary Na >200mmol/24 hr) for Baseline Visit 1 and 12 Month Visit. Urine pregnancy test for females as appropriate.
3) Standardized lifestyle counseling as per goals derived from the AACE and NCEP-ATP III guidelines and modeled after the Diabetes Prevention Program.
4) Coronary CTA only at the end of study visit prior to 12 Months. (This will not be performed if the subject has a previous reaction or contraindication to iodine-containing contrast media).
5) Optional Consent for substudy.

Baseline Visit 1 (Imaging): Subjects will present after a 12 hr fast on 2 consecutive days. CCB will be held 36 hrs prior to coronary perfusion assessment.
1) 24 hr urine will begin on the morning of this visit for volume, Na, K, Cr, cortisol and aldosterone excretion rate.
2) STAT serum pregnancy test for females as appropriate.
3) Urine Na and Cr for sodium balance.
4) Interval history and physical, including orthostatics.
5) Cardiac PET to assess CFR.
6) Subjects will begin fast and lie supine overnight.
7) Fasting blood for PRA, serum aldosterone, K. DNA collection for genetic testing is optional.
8) Cardiac MRI for ECV, endo to epicardial flow (perfusion) ratio, diastolic and systolic strains and peak strain rates, LVEF and LV mass.
9) Abdominal MRI for VAT and SAT.

Baseline Visit 2 (RAAS Characterization): After Baseline Visit 1, subjects will be placed on an isocaloric low sodium diet for 6 days prepared by the metabolic phenotyping core at the BWH Center for Clinical Investigation for assessment of RAAS activation. Subjects will be asked only to consume the prescribed diet, containing 10±2 mEq Na^+, 100±2 mEq K^+, and 1000±50 mg Ca^{2+} per 24 hours.
1) 24 hr urine will begin on the morning of this visit for volume, Na, K, Cr, cortisol, and aldosterone excretion rate.
2) Urine pregnancy test for females as appropriate.
3) Urine Na and Cr (rescheduled if subject is not in sodium balance).
4) Interval history and physical, including orthostatics.
5) Subjects will begin fast and lie supine overnight.
6) Fasting blood for PRA, serum aldosterone, K. DNA collection for genetic testing is optional.
7) Resume ad lib diet and administer study medication (eplerenone vs. placebo) to be started on the following day.

Substudy Visit 1 (Optional): Subjects will present on a low carbohydrate diet, after an overnight 12 hr fast. We will obtain:
1) STAT serum pregnancy test for females as appropriate.
2) Cardiac ^{18}F-FDG-PET/CT to assess arterial inflammation.

Randomization and Blinding: Subjects will be randomized 1:1 to receive eplerenone

50mg daily or identical placebo for 12 months. The randomization list, stratified by gender, statin use and presence of plaque will be generated by the biostatistician and kept by the research pharmacy. Daily dosing starts at 50 mg daily for the first week to avoid hypotension and will be increased to 50 mg twice daily at the 1 week safety visit. The study will be double-blinded. All subjects will receive lifestyle modification as described below.

Safety Visits (1 wk, 2 wk, 4 wk, 3 mo, 6 mo, 9 mo post baseline visits): Subjects will only present after an overnight 12 hr fast for the 6 month visit. All safety visits include:
1) Urine pregnancy test for females.
2) Interval history and physical, including vitals.
3) Blood for Cr, K. Blood for markers of heart disease and inflammation for the 6 month visit only.
4) Collection of used study drug and provision of new supply.
5) Standardized lifestyle counseling as per goals derived from the AACE and NCEP-ATP III guidelines and modeled after the Diabetes Prevention Program. At the 1 week visit, the study dose will be escalated to 50mg twice daily as tolerated based on blood pressure and labs.

12 Month Visit: Same as Baseline Visit 1

Substudy Visit 2 (Optional): Same as Substudy Visit 1

COVID19 Contingency Plan: MGH and BWH have instituted restricted visits for research purposes in response to the evolving COVID-19. These restrictions have prompted the following changes to the study:

1) We may conduct visits remotely until restrictions are lifted. Remote visits will be conducted over zoom video conferencing or telephone and every attempt will be made to keep the visit schedule the same. Changes may occur to the visit schedule due to factors related to covid19. During these visits, someone from the study staff will contact the subject (via zoom video conferencing or telephone) and may conduct the following research activities:

- Consenting*
- Obtaining information about past and present health
- Questionnaires
- Lifestyle Counseling
- Obtaining information related to safety assessments

2) We may ship study drug directly to the subject.

3) If blood work cannot be obtained at the study site, we may ask the subject to have labs performed locally. We will provide a lab requisition and help locate a facility.

4) We will try to arrange for assessments to occur remotely and continue to follow the visit schedule. Some study procedures require a visit to the study site, for example, imaging procedures. We may provide the subject with additional study drug until we are able to perform the study procedure, and this may extend the visit schedule. If a study procedure requires an in-person visit and cannot be scheduled at the study site due to

COVID19 policies, then the procedure may be omitted.

***COVID19 Contingency Plan for Informed Consent:**

A consent form may be mailed or emailed to the subject and a licensed physician or nurse practitioner will arrange a phone call or zoom video conference with the subject to review the consent form in detail. The consent form will be signed and completed via one of the following options: 1) If the subject has electronic capabilities/internet to use REDCap, the consent form will be electronically signed by both parties and a PDF of the consent form will be available for both the subject and the licensed physician or nurse practitioner, or 2) The subject will sign the consent form during the phone discussion and either mail/email the signed form to study staff or bring the signed form to the next in person visit at which time the consent form will be signed by the licensed physician or nurse practitioner, or 3) To minimize exposure time between the subject and study team, an iPad may be offered to the subject to perform a zoom video conference with the licensed physician or nurse practitioner to discuss the consent form prior to meeting in the same room to sign the consent form. Study procedures will not be performed prior to obtaining informed consent and obtaining a copy of the signed consent form.

Study Procedures

Computed Tomography (CT) Abdomen

Images with be acquired via single-slice acquisition through L4 with tube current of mA, voltage of 80kV and 70mAs, slice thickness 10 mm, and 45 cm field-of-view.

Coronary Computed Tomography Angiography (CTA)

Contrast-enhanced CCTA allows for non-invasive assessment of atherosclerotic plaque burden and morphology. Image acquisition will take place at MGH using the SOMATOM Definition Flash, a dual source 128 detector row CT scanner, or the SOMATOM Force, a dual source 196 detector-row CT scanner (Siemens Healthineers, Forchheim, Germany). Semiautomated reference tube current selection and exposure control (CAREDose 4D) are tailored to the subject's body size, in conjunction with semiautomated reference tube potential (voltage) selection (CARE kV), with individually adapted exposure settings on a per-patient basis. Prospective ECG-triggered axial-sequential or retrospective ECG-gated spiral with adaptive pulsing scan modes are used based on the heart rate and rhythm. Iterative image reconstruction algorithms allow for further reductions in radiation dose.

The CCTA protocol is as follows, and is based on the standard clinical CCTA protocol at MGH: Prior to imaging, intravenous (IV) access will have been obtained in an arm or hand vein, usually in the right antecubital vein. The subject's blood pressure will be taken, and ECG leads will be attached to monitor heart rate and rhythm. Sublingual nitroglycerin (0.3-0.9 mg, typically 0.6 mg) or transdermal nitroglycerin (0.6-0.8 mg/hr for ~45 minutes) will also be administered as per standard protocol for cardiac CT scans. Contraindications to nitroglycerin include known narrow angle glaucoma, hypotension with systolic blood pressure < 90 mm Hg, known severe aortic stenosis, or use of phosphodiesterase type 5 inhibitor (e.g. sildenafil, tadalafil, vardenafil) within the 5 days prior to scan. A prospectively-ECG triggered noncontrast CT of the heart is obtained to measure coronary calcium. The test bolus method will be used to plan the CTA timing, via a test bolus injection of nonionic iodinated contrast medium (approximately 20 ml), followed by a flush of saline solution (approximately 40 ml). Contrast-enhanced CT

acquisition will then take place. Based on subject size, 60-100 ml of nonionic iodinated contrast (iopamidol 370 g/cm3, Isovue 370, Bracco Diagnostics, Princeton, NJ) will be injected, followed by a flush of 40 ml of saline solution. After a delay based on the test bolus, a CT acquisition will be performed from the aortic arch to the diaphragm: tube voltage 70 - 120 kVp, tube current-time product reference 320 mAs, detector collimation 2 x 128 x 0.6 mm or 2 x 196 x 0.6 mm. A number of high-resolution images of the heart at slightly different time-positions within the cardiac cycle will be reconstructed, of which the best phase will be used to evaluate the coronary artery lumen, plaque distribution, and plaque morphology.

Cardiac Positron Emission Tomography (PET)

Regional and global coronary blood flow will be assessed using PET imaging. Vasoactive medications and beta-blockers will be withheld on the morning of the study. Subjects will be allowed to continue using nitroglycerin as needed. Studies will be performed after at least 4 hours of fasting. The protocol will take approximately 2.5 hours, including patient preparation. Regional coronary blood flow will be measured at rest and during an intravenous (IV) infusion of regadenoson (0.4mg) or adenosine (0.14 mg/kg/min) using 13N-ammonia as a flow tracer. After IV administration of 13N-ammonia (20 mCi), serial images will be acquired for 20 min, as previously described. After 30 min, IV regadenoson or adenosine will be infused for 4 minutes. Two minutes into the regadenoson or adenosine infusion, a second dose of 13N-ammonia (20mCi) will be injected and images recorded again in the same sequence. HR, BP, and 12-lead ECG will be recorded at baseline and every minute during and after the infusion of regadenoson or adenosine. All images will be analyzed by an expert in cardiac PET blinded to treatment assignment.

Cardiac Magnetic Resonance Imaging (MRI)

T1 imaging before and after contrast, using a breath-hold, modified Look- Locker imaging (MOLLI) technique, will be used for mapping of the total and segmental extracellular volume fraction to detect expansion of the extracellular matrix. Typical MOLLI acquisition parameters are: TR/TE=2.14/1.07 ms, flip angle=35º, FOV= 340x255mm, matrix=192x144, 107 phase encoding steps, interpolated voxel size= 0.9x0.9x8mm, parallel acceleration factor=2 with 24 reference lines (iPAD), cardiac trigger delay TD=500ms; 206 ms acquisition time per SSFP image read-out. The pre-contrast T1 mapping will use a 5(2)-3 MOLLI scheme, while the post-contrast T1 measurements will use a 4(2)-3(2)-3(2) scheme, where the numbers in brackets indicate the duration of the rest period as a multiple of the R-to-R duration. The post-contrast T1 measurements are interleaved with the other MR protocol components, such that T1 measurements are not performed within less than 5 minutes of a contrast injection to assure contrast-equilibrium between tissue and blood. Multiple T1 measurements are used with regression analysis to obtain an accurate estimate of extracellular volume (ECV) fraction, thereby improving upon the precision of “two-point” measurement protocols.[66] Only R1 data-pairs with R1 in blood < 3.5 s-1 will be used to meet the conditions of fast transcytolemmal water exchange, underpinning the linear model for ECV. [67] ECV will be multiplied by LV mass index and reported as ECM*i* (g/m^2). The T1 protocol comprises one pre-contrast measurement, and 3 post contrast measurements as outlined above. Microvascular coronary function via a combination rest/stress MRI perfusion study will be quantified. Study subjects will be administered regadenoson (5 ml containing 0.4 mg regadenoson bolus via IV, followed by flush) or adenosine (400 microgram bolus via IV, followed by flush). A saturation-recovery prepared fast

"TurboFLASH" gradient echo-technique, with iPAT (with mSENSE) acceleration (x2) will be used to measure perfusion at rest and after vasodilatory stress in three slices (basal, mid, apical levels) with an in-plane resolution of 2.2 x 2.2 mm in three short-axis slices, using TR/TE/flip = 2.3/1.0/18°; 10 msec delay after saturation before image read-out; linear phase-encoding order. In addition, a low-resolution TurboFLASH image is acquired within 50 ms during each heart-beat at the basal level. This low resolution image has a T1-weighting that allows to measure the arterial input function (AIF) without significant signal saturation[68]. (Siemens software patch WIP578 for *syngo* MR B17). The saturation-recovery time for the AIF readout is kept to < 20 ms. The software also automatically generates motion-corrected images (in addition to the "raw" images), which substantially reduces the post-processing time for quantification of myocardial perfusion (reserve). A contrast bolus dosage of 0.06 mmol of Dotarem (gadoterate meglumine, Guerbet,Inc.) is injected to allow coronary blood flow quantification using the arterial input measured with the low-resolution AIF "scout", and keeping arterial *and* myocardial signal saturation to < 5%. (We use a dosage of 0.06 mmol/kg for this reason, rather than 0.1 mmol/kg used by Gatehouse et al[68], though this dosage is fully adequate for analyzing myocardial contrast enhancement.). Rest perfusion imaging will be performed > 20 minutes after stress perfusion imaging. We will quantify coronary blood flow, as we have done previously.[69,70] The rationale for acquiring perfusion images with MRI in parallel with the PET studies, is the higher spatial resolution of MRI compared to PET to assess myocardial blood flow in the endocardial layer, and also determine the ratio of endo- to epi-cardial flows, as a marker of ischemia. For post-processing, the perfusion images will be segmented along the endo- and epi-cardial borders of the LV, and a reference point will be placed at the anterior LV/RV junction. Coronary blood flow will be quantified by model-independent deconvolution of the myocardial signal curves with the AIF. Myocardial signal curves for endo- and epicardial layers in 16 segments of the AHA segmentation model will be analyzed. All images will be analyzed by an expert in cardiac MRI blinded to treatment assignment.

Abdominal MRI

Images will be acquired using an axial T1-weighted fat suppressed pulse sequence obtained at the level of L4 vertebral body. Abdominal visceral and subcutaneous fat areas from MRI and CT are determined based on offline analysis of tracings obtained utilizing commercial software (Vitrak, Merge e/Film).

Nutritional Analysis and Dietary Protocol

Protein, carbohydrate, fat, micronutrient, dietary supplements and alcohol intake will be determined from 4-day food records (Nutrition Data systems). For 6 days prior to Baseline Visit 2 (RAAS characterization), subjects will be provided low sodium meals and snacks (10 mEq daily sodium, 100 mEq potassium, 1000 mg calcium, water intake ad lib) and they will be asked not to consume any food not provided to them, including no caffeine or alcohol. For 3 days prior to Baseline Visit 1 and 12 Month Visit, subjects will be provided sodium supplements through broth packets as needed to achieve urinary Na >200mmol/24 hr, and they will be asked not to consume any food not provided to them, including no caffeine or alcohol. Urine aliquots for sodium and creatinine and 24hr urine sodium will be measured for the period immediately prior to each visit to ensure compliance with the diet.

Lifestyle Modification

Goals derived from the AACE and NCEP-ATP III guidelines and the Diabetes Prevention Program are as follows: ≤35% calories from fat, < 7% calories from saturated fat, up to

10% calories from polyunsaturated fat, reduction of trans fatty acid intake, up to 20% calories from monounsaturated fat, and 25-35g of fiber per day. 3 hrs of physical activity/week at moderate intensity. The curriculum is modeled after the Diabetes Prevention Program. Subjects will complete lifestyle sessions at the Clinical Research Center at MGH with nutritionists trained to implement the curriculum. The curriculum will be introduced and reinforced until the completion of the study.

Optional Substudy:
Cardiac 2-deoxy-2-18F-Fluorodeoxyglucose Positron Emission Tomography/ Computed Tomography (^{18}F-FDG-PET/CT)

An approach to detecting macrophage activity in atherosclerotic plaques uses FDG as the contrast radiotracer for positron emission tomography (PET). FDG competes with glucose for uptake into metabolically active cells, including macrophages in atherosclerotic plaques, and is trapped inside cells after phosphorylation. FDG-PET can image metabolic activity (as a marker of plaque inflammation) quantitatively in atherosclerotic plaques[71,72], has been shown to have high reproducibility[73], and correlates with histologic data[72]. Taking advantage of the myocardium's preference for free fatty acids over glucose as an energy source, to minimize myocardial glucose uptake in order to better visualize coronary plaque activity, patients will be asked to adhere to an Atkins style diet for the evening prior to imaging and then to fast overnight. A fasting glucose will be checked prior to the start of the study. Cardiac PET/CT imaging will be performed two hours following intravenous administration of approximately 10mCi of ^{18}F-FDG. Imaging will be performed using a Siemens Biograph PET/CT system (Siemens Medical Solutions, Forchheim, Germany). Semi automated co-registration of PET and CT images will be accomplished using the Siemens MultiModality WorkPlace TrueD (Forchheim, Germany). Once co-registered, the standardized uptake value (SUV) of FDG will be measured in various arteries. The SUV is the decay-corrected tissue concentration of FDG (in kBq/ml) divided by the injected dose per body weight (kBq/g). Target-to-background ratio (TBR) will be calculated for each vascular segment as the segment SUV divided by vena cava or atrial blood SUV. All images will be analyzed by an expert in cardiac FDG-PET/CT blinded to treatment assignment.

VI. Biostatistical Analysis

Statistical Consideration: **Aim I:** The primary endpoint is change in CFR assessed by cardiac PET or change in sMBF by cardiac MRI. Secondary endpoints will include the following: change in sICAM and sVCAM. Shapiro-Wilk test will be used to assess for normality of distribution, and variables that are not normally distributed will be appropriately transformed prior to inclusion in multivariable models. We will compare the change in CFR from months 0 to 12 between treatment groups (eplerenone vs. placebo) using student's t-test for variables that are normally distributed or Wilcoxon rank sum test for variables that are not normally distributed. We will also compare changes in sICAM and sVCAM over 12 months between treatment groups using similar statistical methods. In all Aims, for subjects who withdraw after 6 months, but before the final 12 month visit, we will obtain biomarkers and imaging similar to that scheduled for the final 12 month visit if the subject agrees to have end of study testing. The analysis will be intention to treat (ITT). In order to properly handle subjects with missing outcomes (those who withdrew before 12 months), we will apply longitudinal linear mixed effects model analysis with random subject level intercepts and time slopes by which the longitudinal treatment effect can be examined by testing the significance of

time x treatment interaction, which will provide unbiased effect estimate under missing at random condition, which will be carried out by using augmented data sets created by multiple imputation. A sensitivity analysis will be performed to include only those subjects who completed the study. Baseline variables will be compared between treatment groups, and any variable that is statistically different between groups will be controlled for using multivariable modeling. It may be that subjects with more significant RAAS activation and dysregulation in aldosterone at baseline) are more responsive to eplerenone. We will phenotype the RAAS in subjects at the low sodium visit and, guided by this data, may use aldosterone levels prior to intervention as an index of RAAS activation and a covariate in assessing treatment response, to determine if eplerenone is more effective in the subpopulation with greater baseline aldosterone dysregulation and RAAS activation. Covariates that will be considered for inclusion in modeling, include variables that differ at baseline and those variables that may affect vascular dysfunction, inflammation, fibrosis and plaque (age, race, gender, BP, tobacco/illicit drug use, family history of CVD, lipids, and glucose), HIV parameters (viral load, CD4, and duration of HIV and ART use) and compliance. This will permit us to assess relevant factors that may mediate the effect of eplerenone on CFR in HIV and interactions between HIV and traditional risk factors contributing to eplerenone effects. Moreover, we will compare changes in CFR and vascular biomarkers over 12 months using regression analysis. We will perform Pearson's correlation for variables that are normally distributed and Spearman's correlation for variables that are non-normally distributed. If we see significant changes in specific biomarkers that correlate with changes in CFR, then we will run those specific biomarkers from stored serum at earlier time points (e.g. 6 months) to see if early changes in biomarkers can predict changes in CFR, as well as predict response to MR blockade. **Biostatistician Role:** The biostatistician will monitor data collection, quality and completeness of the study throughout all years of the grant. The biostatistician will create the randomization plan, using a permuted block algorithm, stratified for gender, statin use, and presence of plaque and provide the list to the study pharmacist (investigators will remain blinded). Identical placebo will be provided by the pharmacy. **Sample Size Determination:** Preliminary data from HIV subjects presented in this grant demonstrate a CFR 1.74±0.73 (mean±SD) using cardiac PET Supportive data are also derived from a longitudinal study by our team using cardiac PET, showing mean±SD change of CFR in diabetics treated with spironolactone 0.33±0.83 vs. placebo: 0.02±1.03 over 6 months (mean SD=0.93).[40] Given these data, we considered the range 0.73-0.93 SD to accurately reflect the potential variability of change in CFR for the proposed study. Based on this data, we now provide a range of detectable differences using 85% power and a revised increased sample size of 80 subjects, with an attrition rate of 15%, leaving 68 evaluable subjects. Thus, even choosing a conservative SD of 0.93, the study will be well powered to detect a difference in mean change of CFR of at least 0.69. As our preliminary data, using PET, show that HIV patients have a mean CFR 1.7, an increase of CFR to 2.4 with eplerenone is a clinically relevant increase associated with decreased morbidity and mortality. For the sMBF and biomarkers, this study has 85% power at a two-sided 0.05 α level to show a change in the variable of at least 0.74 SD after treatment with eplerenone vs. placebo.

Aim II: For this aim, the endpoint is change in myocardial ECV assessed by myocardial T1 mapping on cardiac MRI. Secondary endpoints will include the following: change in plasma IL-6, MCP-1, sCD163, sCD14 and serum hsCRP, galectin-3, cardiotrophin 1, lipocalin, PINP, PIIINP, BNP. Statistical algorithms as detailed in Aim I will be applied to

these specific endpoints. Similar to Aim I, changes in ECV and inflammatory/fibrosis biomarkers over 12 mos comparing eplerenone vs. placebo will be assessed using ITT and longitudinal linear effects modeling with imputation and sensitivity analyses. By regression techniques and covariate analyses, we will also assess whether improvements in ECV from MR blockade will relate to improvements in diastolic strain. **Sample Size Determination:** Thiara et al. show mean±SD ECV 0.28±0.04 vs. 0.26±0.03 in HIV vs. non-HIV using cardiac MRI.[30] To estimate the SD of change in ECV, we assumed an intrasubject longitudinal correlation over time r=0.7 among the HIV group, which results in a conservative SD estimate of 0.03. Based on this SD, the proposed study, with a revised sample size of 80 subjects and therefore 68 evaluable subjects considering a 15% attrition rate, will attain 85% power at a two sided α of 0.05 to detect a difference of at least 0.02 in longitudinal means of ECV. Thus our study is well powered to find a significant difference between arms that is clinically relevant, as each 0.03 increase in ECV raises the risk for mortality or CV morbidity by 50%.[23] For biomarkers, we have 85% power at a two-sided 0.05 α level to show a change of at least 0.74 SD comparing eplerenone vs. placebo.

Aim III: For this aim, the endpoint is change in noncalcified plaque volume assessed by coronary CTA. Secondary endpoints will include: change in high risk plaque morphology (numbers of low attenuation and positively remodeled plaque) by coronary CTA. Statistical algorithms as detailed in Aim I will be applied to these specific endpoints. Moreover, the relationship between baseline CFR and non-calcified plaque volume will be assessed. Lastly, we will also determine whether longitudinal changes in coronary plaque are associated with changes in biomarkers of inflammation and immune activation to understand the potential relationship of these inflammatory indices as markers of improved indices of the coronary vasculature in response to MR blockade in the HIV. **Sample Size Determination:** In our prior studies assessing plaque in HIV, the median [IQR] change of noncalcified plaque volume over 12 mos was 6.7[-6.5, 29.8] mm^3.[13] We assumed a conservative SD of 18mm^3 taking the mean of the IQR. This study enrolling 80 subjects with 68 evaluable subjects will attain 85% power at a two sided α of 0.05 to detect a difference in noncalcified plaque volume of 13.3mm^3 between eplerenone vs. placebo, a clinically relevant change. For biomarkers, this study has 85% power at a two-sided 0.05 α level to show a change of at least 0.74 SD after treatment with eplerenone vs. placebo.

Aim IV: For this aim, the endpoint is change in MCP-1 between the low sodium and liberal sodium diets. Secondary endpoints will include: change in hsCRP, pentraxin3, IL6, Lp-PLA_2, sCD14, sCD163. We will test the change in biomarkers between the RAAS activated (sodium restricted) and RAAS suppressed states (sodium loaded) using the paired Student's t-test for normally distributed variables or Wilcoxon signed-rank test for non-normally distributed variables. We will apply Pearson's correlation for variables that are normally distributed and Spearman's correlation for variables that are non-normally distributed to determine whether there is a significant correlation between aldosterone during the RAAS activated state and TBR. Covariates considered for inclusion in modeling include variables that may affect vascular dysfunction, inflammation, and plaque (age, race, sex, BP, tobacco/illicit drug use, family history of CVD, lipids, glucose), and HIV parameters (viral load, CD4, duration of HIV and ART use). This will permit us to assess relevant factors that may mediate the effect of aldosterone on inflammation in HIV and interactions between HIV and traditional risk factors contributing to inflammation. **Sample Size Determination:** Preliminary data in HIV presented in this

grant demonstrate a mean change in MCP-1 of 44.0±41.8 pg/mL between a low sodium and ad lib sodium diet. Based on this SD, the proposed study, with a sample size of 42 subjects, and therefore 36 evaluable subjects considering a 15% attrition rate, will attain 85% power at a two sided α of 0.05 to detect a difference of at least 44.0 pg/mL in mean MCP-1 between the formally tested RAAS activated (sodium restricted) and RAAS suppressed (sodium loaded) states. For other biomarkers, we have 85% power at a two-sided 0.05 α level to show a change of at least 0.95 SD between RAAS activated and suppressed states.

Aim V: For this aim, the endpoint is change in TBR assessed by cardiac ^{18}F-FDG-PET/CT. Secondary endpoints include: change in hsCRP, pentraxin3, IL6, Lp-PLA2, MCP-1, sCD14, sCD163. Statistical algorithms as detailed in Aim I will be applied to these specific endpoints. Similar to Aim I, changes in TBR and inflammatory biomarkers over 12 mos comparing eplerenone vs. placebo will be assessed using ITT and longitudinal linear effects modeling with imputation and sensitivity analyses. We will also determine whether longitudinal changes in arterial inflammation are associated with changes in biomarkers of inflammation and immune activation to understand the potential relationship of these inflammatory indices as markers of improved indices of the coronary vasculature in response to MR blockade in the HIV. **Sample Size Determination:** In our prior studies assessing arterial inflammation on ^{18}F-FDG PET, the mean±SD (95% CI) TBR at baseline among HIV subjects was 2.23 ± 0.45 (2.07, 2.40)mm^3.[74] In order to estimate the SD of change in TBR, we assumed the intrasubject longitudinal correlation over time r=0.75, which results in a conservative SD estimate of 0.34.This study enrolling 42 subjects with 36 evaluable subjects will attain 85% power at a two sided α of 0.05 to detect a difference in mean TBR changes of 0.32 between eplerenone vs. placebo, a clinically relevant difference in the changes as our preliminary data show that non-HIV subjects have a mean TBR 1.89. For biomarkers, this study has 85% power at a two-sided 0.05 α level to show a mean change of at least 0.95 SD after treatment with eplerenone vs. placebo.

Aim VI: In an exploratory analysis, subjects will be enrolled using a factorial sampling strategy into 4 analytic strata (low VAT/absence of plaque, low VAT/presence of plaque, high VAT/absence of plaque, high VAT/presence of plaque). Using this design, we will first analyze whether there are differences in markers of inflammation and heart disease by VAT status (main effects model comparing presence and absence of excess VAT) using student's t-test for variables that are normally distributed or Wilcoxon rank sum test for variables that are not normally distributed. Based on the exclusion criteria excess VAT is determined to be >110cm2. After assessing for differences in VAT in the main effects model, we will examine the degree to which markers of inflammation and heart disease are related to coronary plaque in both the high and low VAT groups, and whether this relationship is stronger in the coronary plaque group as a result of increased VAT using ANOVA for variable that are normally distributed and Kruskal-Wallis Test for variables that are not normally distributed.

VII. Risks and Discomforts

Blood Drawing: The total blood drawn for subjects completing the study is equivalent to approximately 560 ml (over a period of 12 months). This quantity of blood drawing does not pose excessive risk to subjects. By comparison, the Red Cross allows a healthy adult to donate 1 pint of blood every 8 weeks, which is about 473 ml. Subjects with a hemoglobin < 10 mg/dL will be excluded from the study. There will be minimal risk and

discomfort associated with blood drawing and IV placement. The risks of these procedures are minor bruising or bleeding at the site of the blood draw or IV catheter or feeling faint. Standard clean insertion techniques will be utilized to minimize risk of infection.

Intravenous Catheter Placement: There are minor risks and discomforts associated with having an intravenous line and with injection of contrast agent through the intravenous line, including the possibility of bleeding, pain, inflammation (redness and swelling), and leaking of contrast agent outside of the vein at the site of the IV. These problems usually self-resolve or only require minor treatments, such as application of an ice-pack or slight pressure for a few minutes.

CT Abdomen: This study is obtained once during the screening process to evaluate for visceral adipose tissue area. The radiation risk associated with the single slice CT scan of the abdomen is approximately 0.55 mSv. This is equivalent to the amount of radiation an individual would normally get in 2 months from natural background sources from the earth and the sky. As with the other imaging studies, there may be incidental findings on the CT study. If any of these findings are clinically significant, the subjects will be withdrawn from the study and referred to the primary care physician for further evaluation.

Coronary CTA and Radiation: With the implementation of measures to limit radiation exposure, the total dose of the coronary CTA examination will be approximately 4 mSv, depending on the weight, gender and heart rate. The added radiation from the two CTA scans (screening and follow-up) will be 8 mSv. This is equivalent to the amount of radiation an individual would normally get in 2 years and 7 months from natural background sources from the earth and the sky. State-of-the-art dose reduction techniques at MGH allow the dose to be substantially lower than historically reported median coronary CTA doses. Iterative image reconstruction algorithms allow for further reductions in radiation dose. These techniques allow for up to a 50% reduction in radiation dose compared with previous generation protocols. Massachusetts General Hospital was the first hospital in New England to use the Siemens Definition Flash second-generation dual-source 128 or Siemens SOMATOM Force dual-source 196 detector-row CT, an advanced cardiac-capable CT scanner which allows ultra-fast cardiac imaging at the lowest possible radiation doses potentially equivalent to or less than a chest CT scan. HIV patients may have clinically significant subclinical CAD. We will assess the first coronary CTA for this condition. Any subject with evidence of severe coronary artery stenosis, defined as ≥50% stenosis in the left main coronary artery or >70% stenosis in the other major coronary arteries will be withdrawn from the study prior to further evaluative imaging and procedures and prior to randomization to study drug. Subjects with evidence of clinically significant atherosclerosis will be referred to a cardiologist for appropriate evaluation and treatment.

CTA contrast medium: CTA requires the intravenous administration of iodinated contrast medium. These contrast media have been known to cause renal failure, particularly in subjects with a pre-existing renal impairment. Adequate hydration will also be emphasized to each study participant. The contrast dose in coronary CTA has decreased with the introduction of faster scanners. The dose required will be between 60 to 100 ml. Allergic reactions to iodinated contrast media may cause skin reactions or in very rare occasions result in breathing difficulty and hypotension. Subjects will be closely

monitored for the development of any allergic reactions. To minimize risk from iodinated contrast, subjects with previous allergic reactions to iodine-containing contrast media or signs of renal failure or history of renal disease will be excluded from the CTA study. Additional safety criteria will be implemented by measuring serum creatinine and excluding subjects with serum creatinine >1.5 mg/ml or estimated GFR<60 mL/min/1.73m^2. Metformin use is an exclusion criteria for the study, so the risk of lactic acidosis will be avoided.

Nitroglycerin: Nitroglycerin is commonly used in subjects with angina pectoris and is generally considered safe. The side effects and risks of nitroglycerin are generally mild and of short duration and include hypotension, tachyarrhythmia, headache, lightheadedness, and visual disturbance. Subjects who are on sildenafil, tadalafil, or vardenafil (PDE5 inhibitor) will need to stop these drugs at least 5 days prior to receiving nitroglycerin on the day of the coronary CTA scan. If baseline systolic blood pressure is below 90, nitroglycerin will not be given. If a participant's blood pressure were to drop precipitously or if the participant develops symptoms, standard clinical care will be provided. Other contraindications to nitroglycerin include severe aortic stenosis or hypertrophic obstructive cardiomyopathy.

Cardiac PET and Radiation: The two cardiac PET scans will involve exposure to radiation for research purposes. Subjects will receive a research related rest and stress ^{13}N-ammonia PET scan before and after a mineralocorticoid receptor antagonist. ^{13}N-ammonia (20 mCi) will be injected twice prior to rest and stress scans. Each PET scan will expose subjects to 3.36 mSv of radiation. The added radiation from the two PET scans (baseline and follow-up) will be 6.72 mSv. This is equivalent to the amount of radiation an individual would normally get in 2 years and 2 months from natural background sources from the earth and the sky. The radioactive tracer will be administered at baseline and at 12 months. HIV patients may have silent ischemia. We will assess the first regadenoson/adenosine-stimulated coronary blood flow study for this condition. Any subject with evidence of clinical significant ischemia will be withdrawn from the study prior to further evaluative studies and prior to randomization to study drugs. Subjects with evidence of ischemia will be referred to a cardiologist for appropriate evaluation and treatment. In addition, there may be incidental findings on the PET study. If any of these findings are clinically significant, the subjects will be withdrawn from the study and referred to the primary care physician for further evaluation.

Regadenoson/Adenosine: Regadenoson or adenosine will be administered during the cardiac PET and MRI studies. Regadenoson stress and adenosine stress have been used routinely for many years for evaluating patients with known or suspected CAD. Regadenoson and/or adenosine will be administered at the radiologist's discretion. The most common side effects associated with the Regadenoson or adenosine infusion include: flushing, chest pain/pressure, shortness of breath, palpitations, headache, mild hypotension and heart block. These side effects are usually mild and self-limiting. If they are severe in intensity, aminophylline IV (1 mg/kg) will be given as per standard protocol. Subjects will have their blood pressure and heart rate monitored closely by a physician and/or nurse in attendance at all times. The protocol will be immediately discontinued if clinically significant atrial or ventricular arrhythmias are observed.

Radiation Exposure: The total estimated radiation exposure from Aims 1 and 3 is approximately 15.27 mSv. Aim 2 has no specific radiation associated. This is equivalent to the amount of radiation an individual would normally get in 4 years and 11 months from natural background sources from the earth and the sky. To minimize radiation exposure, coronary CTA dose reduction techniques including ECG-triggered tube modulation and automatic exposure control will be applied when possible. Larger patients can get higher doses (up to twice that of an average size patient) from any CT exam compared to the mean dose. If a patient has received previous radiation exposure prior to participation in the study, we will also calculate the total radiation exposure and if prior cumulative exposure during the 12 months prior to the study exceeds 15 mSv (which is 30% of the maximum recommended annual dose for research subjects set by the MGH Radiation Safety Committee), then the subject will be excluded from participating. Pregnancy test will be performed prior to CT or PET scanning in women. (See also sections for coronary CTA and cardiac PET above for specific radiation exposure.)

Abdominal MRI and Cardiac MRI: Subjects will be carefully screened for metal implants such as surgical clips or pacemakers prior to MRI scanning. Sources of discomfort during the MRI study may include noise and possible claustrophobia. Subjects will be given earplugs due to the loud noises during the test. Subjects who feel uncomfortable in confined spaces may have difficulty in the narrow cylinder of the MRI, and the MRI can be stopped at any time at the subject's request. There are no known hazards for pregnant women; however, pregnancy is an exclusion for this study. The MRI has the potential, during normal routine use, to cause localized warming of the skin and the underlying tissues. If the subject experiences discomfort due to warming, the procedure will be stopped. Radio-frequency power deposition is strictly controlled by the scanner software, in conformance with the applicable FDA guidelines, and cannot be overridden by the user. An IV line, placed just prior to the MRI exam, may cause tenderness, swelling, warmth at the injection site, and rarely infection. There is no radiation exposure other than exposure to the magnetic and non-ionizing radio-frequency fields, which meet FDA guidelines. All pulse sequences used for this study have been approved by the hardware vendor for cardiac MRI studies, and received FDA clearance, except for the sequence that is used for perfusion imaging, which is a "works-in-progress" package that has undergone safety testing by Siemens Medical Systems, and been licensed for use to Brigham and Women's Hospital. A single axial slice of the abdomen will be obtained with MRI. The total amount of time in the magnet will be approximately 45 min. For the cardiac MRI, the total amount of time in the magnet will be approximately 80 min. In addition, there may be incidental findings on the MRI study. If any of these findings are clinically significant, the subjects will be withdrawn from the study and referred to the primary care physician for further evaluation.

Gadolinium Contrast: Gadolinium contrast has been widely used and is very well tolerated. Gadolinium will be administered with the cardiac MRI studies. No contrast is administered with the abdomen MRI. The most common adverse reactions of gadolinium are transient headache (3.6%), injection site coldness (3.6%), and nausea (1.5%). There is a very rare occurrence of nephrogenic systemic fibrosis after gadolinium, but this has only been reported in subjects with impaired renal function. All subjects will have a serum creatinine assessed prior to each MRI and subjects with serum creatinine >1.5 mg/ml or estimated GFR<60 will be excluded from the study. For this study a macrocyclic Gd-contrast agent will be used (Gadoterate meglumine, DOTAREM,

Guerbet) which has the highest thermodynamic stability constant among all available gadolinium-bound contrast agents to reduce the chance of any exposure to Gd^{3+} ions.

Incidental Findings: Incidental non-coronary and coronary findings may be obtained, and significant non-coronary and coronary findings will be communicated to the subject and provider as appropriate.

Standardized High Sodium Diet: Select subjects will be receive broth packets for 3 days. Increases in dietary sodium may cause mild elevations in blood pressure, but usually only by a small amount depending upon the subjects' salt-sensitivity status. Blood pressure will be monitored frequently during study visits. Procedures will not be initiated if the subject's blood pressure is too high or symptoms are present. When subjects resume their usual home diets, blood pressure is expected to return to baseline.

Medications: Administration of all medications will be conducted by the appropriate health care professional. A physician will be available on-call 24 hours/day, 7 days/week, to all study participants for any questions or concerns.

Eplerenone: The eplerenone dose of 50mg daily has been used safely in previous studies to improve coronary circulation in individuals with Type 2 diabetes and by others in numerous studies of patients with CVD. This dose is FDA approved for indefinite use in patients with hypertension or left-ventricular dysfunction following myocardial infarction. Hyperkalemia is a potential side effect of eplerenone use, and any subject with serum K > 5.5 mEq/L at screen will be excluded. During the study, a subject who has an elevated K of > 5.5 mEq/L at any study visit will have a recheck; if the K remains elevated after recheck, the dose will be decreased to 50mg daily. If the K remains elevated> 5.5 mEq/L, the subject will be excluded from the study. Additionally, subjects who experience symptoms of hyperkalemia and have a K > 5.0 mEq/L will be excluded from continued participation in the study. Subjects who have hypotension (SBP<90 mmHg) at any study visit after titration to full dose eplerenone 50mg twice daily will be given a dose reduction to 50 mg daily. If a subject remains hypotensive (SBP <90 mmHg) or symptomatic of hypotension at the reduced dose they will be dropped from the study. Since subjects with renal insufficiency may require reduced dosing based on creatinine clearance, subjects with serum creatinine > 1.5 mg/dl will also be excluded. Other rare side effects include dizziness, fatigue, and diarrhea; these will be assessed for throughout the study. The effect of eplerenone on an embryo, fetus, or on a breastfeeding infant, is unknown and may be harmful. Because of these unknown risks, women cannot take part in this study if they are: pregnant, trying to become pregnant, breastfeeding. If women become pregnant or think they are pregnant during the study, they must stop taking the study medication and will be withdrawn from the study. We have received FDA IND exemption for the use of eplerenone in HIV for insulin resistance and recently received an FDA IND exemption for application in CVD indices in HIV. Protease inhibitors with the most significant effect on CYP3A4 will be exclusionary as in our prior trials of HIV patients. We will also exclude subjects using other strong inhibitors of CYP3A4, as well as CYP3A4 inducers. Furthermore, all subjects will be carefully monitored for side effects, including frequent potassium and BP monitoring. A 50mg twice daily dose of eplerenone has been chosen, such that even if concentrations do increase somewhat through pharmacokinetic interactions, we expect the drug to be well tolerated. The dose of 100 mg/day has been effective in numerous studies of non-HIV patients and is expected to be effective in the proposed studies.

Optional Substudy--Cardiac 2-deoxy-2-18F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography (^{18}F-FDG-PET/CT) and Radiation:
The two cardiac PET/CT scans will involve exposure to radiation for research purposes. Subjects will receive a scan before and after a mineralocorticoid receptor antagonist. For ^{18}F-FDG-PET/CT, the administered dose from ^{18}F- FDG for a typical cardiac study is 370 MBq (10 mCi=7.03 mSv) and the effective dose for an attenuation correction scan of head/neck/chest/upper abdomen is about 1.58 mSv. The total effective dose from two FDG-PET/CT studies would be 17.22 mSv. This is equivalent to the amount of radiation an individual would normally get in 5 years and 7 months from natural background sources from the earth and the sky. A STAT serum pregnancy test will be performed on the day of prior to commencement of the study. In addition, there may be incidental findings on the PET study. If any of these findings are clinically significant, subjects will be informed, referred to their primary care physician for further evaluation, and withdrawn as appropriate from the study.

Optional Substudy--Radiation Exposure The total estimated radiation exposure for the entire study (main study+substudy) would be 32.49 mSv. This is equivalent to the amount of radiation an individual would normally get in 10 years and 6 months from natural background sources from the earth and the sky. (See also sections radiation exposure for other precautions and cardiac ^{18}F-FDG-PET/CT above for specific radiation exposure related to the substudy.)

Optional Substudy--Blood draw The total amount of blood drawn for the substudy will be approximately 4 mL over 1 year for females only. This will be a minimal amount added to the main study. The total blood drawn for subjects completing the entire study (main study+substudy) is equivalent to approximately 564ml (over a period of 12 months).

Optional Blood Sampling Visit (for those that do not qualify after completion of both screening visits)--Blood draw The total amount of blood drawn for the Optional Blood Sampling Visit will be approximately 48 ml. This will be a minimal amount added to the screening visits. The total blood drawn for subjects completing this optional visit (screening visits+optional visit) is equivalent to approximately 73 ml.

VIII. Potential Benefits

There are no direct benefits related to participation in the study, but subjects will receive regular physical exams with blood pressure monitoring, lifestyle counseling to improve nutrition and physical activity, and screen testing that will evaluate for clinically significant coronary artery disease. There may be potential improvements in CVD and inflammation as a result of treatment with eplerenone and/or lifestyle modification. In addition, all subjects will be provided with tools to help them meet the prescribed goals, including a core curriculum binder. Clinically interpretable information on metabolic parameters will be provided to the subject and may help subjects and their clinicians to better understand their metabolic risk if they choose to obtain such information. Any abnormal testing will be conveyed to the subjects' primary care physicians with their permission. In addition, information obtained from this study will benefit HIV patients in the future. Therefore, the benefits of this study are felt to outweigh its risks described above.

For those who do not qualify and choose to participate in the optional blood sampling visit, there are no direct benefits, but subjects will receive screen tests that will evaluate clinically for visceral fat and coronary artery plaque and inform their medical history. In addition, information obtained from the stored blood will benefit HIV patients in the future. Therefore, the benefits of this study are felt to outweigh its risks described above.

IX. Monitoring and Quality Assurance

Written informed consent for this protocol will be obtained from all subjects at the first screening visit, prior to any procedures or interventions. In all cases, consent will be witnessed by an appropriate health care professional (investigator/co-investigator staff). All efforts will be made to insure the privacy rights of the subject. No written or oral communication will be made about any subject with anyone other than the subject, unless the subject so requests. Medical information obtained from the study may become part of the subject's permanent hospital record, subject to the confidentiality and privacy regulations of the Massachusetts General Hospital. Subjects will be closely monitored to ensure that study participation is not adversely affecting their quality of life, and any adverse events will be reported in a timely fashion and in accordance with the Partners HRC guidelines for adverse event reporting. PHRC requires the reporting of unanticipated problems, including risks, within 5 working days or 7 calendar days. Kidney function and potassium levels will be serially monitored. If a subject experiences an opportunistic infection or any acute illness, the lifestyle modification program will be held while the subject is acutely ill.

Hyperkalemia is a potential side effect of eplerenone use, and contraindications for the use of eplerenone include serum K > 5.5 mEq/L at initiation. Therefore, any subject with serum K >5.5 mEq/L at screen will be excluded from the study. During the study, a subject who has an elevated K of > 5.5 mEq/L at any study visit will have a recheck; if the K remains elevated after recheck they will be excluded from the study. Additionally, subjects who experience symptoms of hyperkalemia and have a K > 5.0 mEq/L will be excluded from continued participation. Subjects who have symptomatic hypotension at any study visit after titration to full dose eplerenone 50mg twice daily will be given a dose reduction to 50 mg daily. Since patients with renal insufficiency may require reduced dosing based on creatinine clearance, subjects with renal insufficiency (serum creatinine > 1.5 mg/dl) will also be excluded from participation. Other side effects have been reported and include dizziness, fatigue, diarrhea, headache, stomach pain, cough, breast enlargement or breast enlargement, rash, abnormal vaginal bleeding. Rare but serious side effects include chest pain, tingling in arms and legs, loss of muscle tone, weakness or heaviness in legs, confusion, lack of energy, cold, gray skin or irregular heartbeat. These potential side effects will be assessed for throughout the study. Exclusion criteria during the study include initiation of ACE-I, ARB, or spironolactone; Hgb < 10; ALT >3.0x ULN; persistent hyperkalemia (K >5.5 mEq/L despite recheck and/or symptoms of hyperkalemia if K >5.0 mEq/L). If a subject remains hypotensive (SBP <90 mmHg) or symptomatic of hypotension after a dose reduction of eplerenone to 50 mg for this same reason during the study, they will be dropped from the study.

Data Safety Monitoring Plan

Prior to the start of study, a data safety monitoring board will be created and this board will consist of a statistician**,** a clinician not directly related to the research project, but one who is familiar with HIV infection and its complications, and a member of the HIV community. This board will meet quarterly to review any potential side effects or adverse events in relation to the use of eplerenone or participation in the study. The

DSMB will meet and review adverse events in accordance with the guidelines of the Massachusetts General Hospital Human Research Committee. Reports generated by the DSMB will be submitted to the Partners Institutional Review Board after when otherwise indicated or requested.

The principal investigator and/or co-investigator will be responsible for following patients during the study. Patients will be assessed at every visit during their study participation. The investigators and study staff will assure that all CRFs, source documents and informed consent are accurate and complete after each study visit.

The data from these studies are stored in a password protected file with access only to study staff and those interpreting the studies. All data are securely stored and only accessible to co-investigators and study staff in order to protect confidentiality.

X. References

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Appendix A

Strong Inhibitors of CYP3A4

- Ketoconazole
- Itraconazole
- Fluconazole
- Ritonavir
- Nelfinavir
- Indinavir
- Clarithromycin
- Telithromycin
- Erythromycin
- Nefazadone
- Bergamottin (in grapefruit juice)
- Quercetin (nutritional supplement)
- Aprepitant
- Verapamil
- Chloramphenicol
- Saquinavir