<u>Supplementary table 1:</u> LFS parameters at baseline and week 96 in HIV-monoinfected and HIV coinfected with hepatitis B or C participants:

	HIV-monoinfected patients			***HIV/HCV or HBC co-infected patients***		
	Baseline (n=128)	***96 weeks (n=104)***	***P value***	***Baseline (n=19)***	***96 weeks (n=15)***	***P value***
Fasting AST (U/dL)	*21.13 ±10.3*	*28.21 ±24.3*	*p<0.001*	*49.0 ±43.0*	*43.7 ±29.4*	*p=0.5*
Fasting ALT (U/dL)	*33.59 ±17.3*	*40.11 ±40.0*	*p=0.08*	*59.16 ±38.6*	*46.1 ±24.7*	*p=0.03*
Fasting insulin(mU/L)	*13.28 ±15.0*	*15.32 ±13.2*	*p=0.03*	*21.0 ±15.7*	*15.4 ± 8.2*	*p=0.01*
Patients with metabolic syndrome	*26 (20%)*	*34 (27%)*	*p<0.001*	*6 (31%)*	*6 (31%)*	*p=0.4*
Liver Fat Score	*-0.4 ±2.6*	*0.23 ±2.6*	*p<0.001*	*1.94 ±3.4*	*0.87 ±2.2*	*p=0.02*

Results reported as mean ± standard deviation for continuous variables and frequency (percent) for categorical variables.
AST, Aspartate aminotransferase; ALT, alanine aminotransferase

<u>Supplementary table 1:</u> LFS parameters at baseline and week 96 in HIV-monoinfected and HIV coinfected with hepatitis B or C participants:

	HIV-monoinfected patients			***HIV/HCV or HBC co-infected patients***		
	Baseline (n=128)	***96 weeks (n=104)***	***P value***	***Baseline (n=19)***	***96 weeks (n=15)***	***P value***
Fasting AST (U/dL)	*21.13 ±10.3*	*28.21 ±24.3*	*p<0.001*	*49.0 ±43.0*	*43.7 ±29.4*	*p=0.5*
Fasting ALT (U/dL)	*33.59 ±17.3*	*40.11 ±40.0*	*p=0.08*	*59.16 ±38.6*	*46.1 ±24.7*	*p=0.03*
Fasting insulin(mU/L)	*13.28 ±15.0*	*15.32 ±13.2*	*p=0.03*	*21.0 ±15.7*	*15.4 ± 8.2*	*p=0.01*
Patients with metabolic syndrome	*26 (20%)*	*34 (27%)*	*p<0.001*	*6 (31%)*	*6 (31%)*	*p=0.4*
Liver Fat Score	*-0.4 ±2.6*	*0.23 ±2.6*	*p<0.001*	*1.94 ±3.4*	*0.87 ±2.2*	*p=0.02*

Results reported as mean ± standard deviation for continuous variables and frequency (percent) for categorical variables.
AST, Aspartate aminotransferase; ALT, alanine aminotransferase

Randomized Placebo-controlled Trial of Rosuvastatin in HIV-infected Subjects to Modulate Cardiovascular Risk and Inflammation

Principal Investigator:
Grace McComsey, M.D.
Professor of Pediatrics and Medicine
Chief, Pediatric Infectious Diseases and Rheumatology
Case Western Reserve University
11100 Euclid Avenue
Cleveland, OH 44106-4984
Phone: (216) 844-3645
FAX: (216) 844-8362
E-Mail:mccomsey.grace@clevelandactu.org

SCHEMA

DESIGN: This is a single-site, randomized, double-blind, placebo-controlled trial of rosuvastatin therapy in HIV-1 infected subjects receiving stable antiretroviral therapy (ART) and who have evidence of heightened immune activation (hsCRP ≥2 mg/L or CD8+ HLA-DR+ CD38+ ≥ 19%) and LDL-cholesterol ≤130 mg/dL. Subjects will be randomized 1:1 in a double-blinded fashion to either 1) Group 1: Rosuvastatin at 10 mg once daily or 2) Group 2: Matching placebo once daily. This study will examine the effect of this intervention on carotid intima media thickness, markers of inflammation and endothelial activation, flow-mediated vasodilation, oxidative stress, bone density and turnover, and lipid and glucose metabolism.

DURATION: 96 weeks.
SAMPLE SIZE: 140 subjects
POPULATION: HIV-infected men and women 18 years of age or older, who are on stable ART for at least 12 weeks, with cumulative prior ART of at least 6 months and plasma HIV-1 RNA level of ≤ 1,000 copies/mL.

REGIMEN: Group 1: Rosuvastatin at 10 mg once daily

Group 2: Matching placebo once daily

In both groups, all ART will continue unchanged, and will not be provided by the study

Patients will be stratified by 1) protease inhibitors (PI) vs. no PI at study entry and 2) by BMD at baseline (t-score <-1 at hip or spine at baseline vs. t-score ≥-1) 3) CT calcium score at baseline (Agatston score 0-99 vs. 100 or above)

1.0. STUDY OBJECTIVES

1.1. Primary Objectives:

- To ascertain whether rosuvastatin affects endothelial function in HIV-infected subjects with good HIV virologic control, we will assess changes overtime in flow-mediated dilation of the brachial artery

- To examine the effect of rosuvastatin on subclinical atherosclerosis, we will assess changes overtime in carotid intima media thickness progression in HIV-infected subjects with good HIV virologic control

1.2. Secondary Objectives:

- To assess the safety and tolerability of 96 weeks of rosuvastatin in aging HIV-infected subjects

- To examine the effect of rosuvastatin on endothelial activation markers, we will assess changes in ICAM-1 and VCAM-1 in HIV-infected subjects with good HIV virologic control
- To examine the correlation between changes in endothelial activation markers and those in FMD and IMT
- To determine if early changes in FMD and endothelial activation markers predict later changes in carotid IMT progression
- To examine the effect of rosuvastatin on changes in inflammation markers and immune activation marlers in HIV-infected subjects with good HIV virologic control, we will assess changes in levels of hsCRP, IL-6, plasma TNF-□, soluble TNF receptors I and II, adiponectin, RANKL, immune activation markers (including expression of CD38 and/or human leucocyte antigen-DR (HLA-DR) on CD8+ lymphocytes), and OPG
- To determine if early changes in inflammation markers predict changes in endothelial function and carotid IMT progression
- To ascertain whether rosuvastatin significantly affect oxidative stress in HIV-infected subjects, we will assess changes overtime in F2-isoprostanes. We will also correlate baseline and changes in F-2 isoprostanes with those of carotid IMT, endothelial and inflammatory markers.
- To examine the effect of rosuvastatin on lipid parameters in HIV-infected subjects with good HIV virologic control, we will assess changes in total-C, LDL-C, HDL-C, total/HDL ratio, and triglycerides
- To examine the effects of rosuvastatin on prevalence of metabolic syndrome and lipid and glucose metabolism in HIV-infected subjects with good virologic control, and correlate these changes with those of IMT, inflammation and endothelial markers
- To examine the effect of rosuvastatin on markers of bone metabolism, we will assess changes in CTX, PINP, and hip and lumbar spine BMD in HIV-infected subjects with good HIV virologic control.
- To correlate changes in inflammation and oxidative markers with those of bone health (BMD and bone turnover markers).
- To examine the effects of rosuvastatin on kruppel-factor like (KLF) 2 and 4, we will assess changes overtime in these markers. We will also correlate baseline and changes in KLF-2 and -4 with those of carotid IMT, calcium scoring, FMD and pro-inflammatory cytokines
- To examine the effect of rosuvastatin on pericardial fat, we will measure change in pericardial fat volume by CT scan.

2.0. Hypotheses to be tested:

We hypothesize that 96-weeks of rosuvastatin is safe and effective in improving endothelial dysfunction, slowing carotid IMT progression, and decreasing inflammation and oxidative stress in HIV-infected ART-treated subjects with good HIV virologic control. Also, we will assess the correlation between these measurements. We will also hypothesize that rosuvastatin will induce beneficial changes in the prevalence of metabolic syndrome and lipid metabolism, will increase BMD and bone formation markers and decrease bone resorption markers.

3. BACKGROUND AND RATIONALE

3.1. HIV-associated cardiovascular disease:

The advent of potent ART drugs in recent years has had an impressive impact on mortality and disease progression in HIV-infected patients. However non-AIDS related events, including cardiovascular disease (CVD) rapidly gained a major importance in their impact on morbidity and mortality of HIV infected subjects treated with ART. Recent analysis on more than 26,000 HIV-infected subjects from 16 cohorts in Europe and North America revealed that the life expectancy of a 20-year-old starting HIV treatment stops short at 58 years (1). The analysis took into consideration a large number of confounding factors. This life expectancy is significantly shorter than that of an HIV-uninfected population residing in these developed countries. Large prospective studies have now demonstrated increased cardiovascular risk in HIV infected patients. However these studies are conflicting regarding the role played by ART versus HIV itself in the generation of the heightened cardiovascular risk. In the DAD study, the risk of myocardial infarction increased by 16% with each additional year of protease inhibitor exposure, independent of traditional risk factors (2). The relative risk of MI was reduced when controlling for lipid levels, suggesting that dyslipidemia played a partial role in the increased risk of MI. Another way to effectively assess CVD risk in HIV is to measure surrogate marker of atherosclerosis, which would become abnormal long before clinical events happen. Carotid intima-media thickness (IMT) is a well-accepted, non-invasive method of monitoring subclinical atherosclerotic formation and progression, and increased IMT is a risk factor for subsequent CVD. Several studies conducted to date demonstrate increased IMT in HIV-infected individuals over healthy controls (3-7), and that IMT progresses more rapidly over time in HIV+ patients (5, 6). The PREVALEAT study demonstrated that carotid IMT was greater among PI-treated patients in comparison with antiretroviral-naive or NNRTI-treated patients. In a case-control study that compared HIV-infected and -uninfected patients matched for age and gender, Hsue et al. reported that HIV infection per se was an independent predictor of IMT and that carotid IMT progresses 10 fold more rapidly in HIV-positive individuals when compared to the historical rate in the general population (5) Johnsen et al. found a similar IMT in HIV-infected and -uninfected women; however, the exposure to PI-containing HAART was strongly associated with an increased IMT (4). On the other hand, some studies suggested that traditional risk factors, and not ART, are major determinants of IMT progression (8). Merciè et al. demonstrated that older age, male gender and smoking are major determinants of IMT progression (6). Currier et al of the AACTG 5078 trial did not confirm the correlation between PI exposure, HIV infection and carotid IMT; in this study, traditional risk factors for atherosclerosis such as low high-density lipoprotein cholesterol, high triglycerides, older age and high BMI were found to be predictive of greater carotid IMT (8). HIV infected subjects in this study had a similar IMT progression overtime than matched HIV negative controls (8), although this study excluded HIV infected subjects with family history of CVD, diabetes and other prevalent metabolic abnormalities associated with HIV. This study population does not mirror the common HIV infected population in clinical practice and thus the AACTG 5178 study results may not reflect the real life HIV population.

Thus, the data from ongoing longitudinal studies with a prolonged follow-up are needed before we can conclude whether HIV infection itself or HAART has a significant role in the occurrence and in the progression of subclinical atherosclerosis. While attempts at clarifying the pathogenesis are underway, the serious consequences of premature atherosclerosis preclude further waiting for an etiology before studying interventions aimed at alleviating this complication.

Increasing evidence suggests that chronic inflammation and endothelial dysfunction may play an important role in the CVD risk, yet their contribution remains incompletely defined. Within HIV, there are studies suggesting that chronic inflammation contributes to the increased cardiovascular risk. Strikingly, recent data from the SMART trial have shown that patients who had an intermittent interruption of their ART had an increased risk of developing cardiovascular disease when compared to those who were on continuous

therapy (9). This finding could be explained by increased immune activation and a heightened inflammatory state in the group whose virus was not continually suppressed with therapy. To further support this hypothesis, Torriani et al found that patients placed on ART had rapid improvement of endothelial function, which has been shown to be an important factor in the development of CAD (10).

3.2. Statins: a potential modulator of cardiovascular risk

3.2.1. Statins and changes in endothelial function

There is increasing evidence that statins exert effects beyond cholesterol lowering. Indeed, many of these cholesterol-independent or "pleiotropic" vascular effects of statins appear to involve restoring or improving endothelial function through increasing the bioavailability of nitric oxide, promoting re-endothelialization, reducing oxidative stress, and inhibiting inflammatory responses (11,12).Thus, the endothelium-dependent effects of statins are thought to contribute to many of the beneficial effects of statin therapy in cardiovascular disease. Interestingly, the beneficial effect of statins on endothelial function has been shown after a short duration of treatment. Twenty-seven elderly diabetic patients with or without mild hypercholesterolemia, were given a low dose of cerivastatin treatment (0.15 mg/d) for 3 days. FMD significantly increased but without an changes in lipid levels (13); however, the oxidant stress 8-oxo dG significantly decreases, suggesting that some of the improvement in endothelial function may be driven by decreasing oxidative stress. Another potential mechanism for lipid-independent improvement of endothelial function, as assessed by FMD, is the increase in the number of circulating endothelial progenitor cells, which is at least partly independent from reduction in plasma cholesterol(14). Overall, the improvement in endothelial function after statins has been shown in multiple non-HIV populations, including patients with hypercholesterolemias (14), nephrotic syndrome (15), and even in healthy offspring of parents with type 2 diabetes mellitus (16).

3.2.2. Effect of statins on inflammation

A potent effect of statins on attenuation of chronic vascular inflammation has been shown outside of HIV and is thought to significantly contribute to the protective cardiovascular effect of statins. In human endothelial cells, statins abrogate TNF-alpha-induced NF-kappaB activation; this occured independently of the classical IKK-pathway but via inhibition of PI3-kinase/Akt signaling (17). Also in cultured human endothelial cells, statins reduced TNF-α-induced osteoprotegerin (OPG) production, and also downregulated interleukin-1α (IL-1α)-induced OPG production in endothelial cells (18). Thus, one possible mechanism for modulation of cardiovascular disease by statins could be regulated by OPG. In clinical trials, as early as 4 weeks of low or high dose of statins have been shown to decrease levels of IL-6, IL-8, CRP, monocyte chemoattractant protein-1, and TNF-□ (16, 19-22). The recently reported JUPITER study perhaps provides the best evidence of the independent role of inflammation in CVD and of the potent beneficial effect of modulating inflammation by statins on cardiovascular disease (23). The JUPITER trial compared rosuvastatin vs placebo in apparently healthy people who had LDL-cholesterol <130 mg/dL but hs-CRP≥2 mg/L. Rosuvastatin treatment lowered LDL-C levels by 50% and hs-CRP levels by 37%, accompanied by a 44% relative risk reduction in the composite end point of unstable angina, revascularization, and confirmed death from cardiovascular causes (23).

3.2.3. Statins and changes in carotid IMT and atherogenesis

It was determined that carotid IMT progression is a good surrogate for cardiovascular

disease endpoints in statin trials (24). Several studies showed regression of carotid IMT overtime in subjects treated with statins in general, (25, 26) and rosuvastatin in particular (27). This regression in IMT was seen as early as 6-12 months after statin therapy. The rate of progression of carotid arterial intima-media thickness in the patients receiving statins varied widely depending on the different studies, IMT method and the population studied, but overall ranged from -0.0014 to -0.0038 mm/year, compared to an increase overtime in the non-statin arm.

Statins and regulation of bone metabolism

Studies outside of HIV have suggested that statins may have a role in regulating bone metabolism and may have a significant effect in maintaining skeletal health. The effect of statins on bone may be multifactorial. Statins stimulate the bone morphogenetic protein-2 (BMP-2), making it a strong stimulator of bone formation (28). Statins increase human osteoblast differentiation as measured by alkaline phosphatase expression and mineralisation (29), or expression of BMP-2 and osteocalcin (30). Statins may also increase bone formation by its proangiogenic effect. Statins produce increased proliferation and differentiation of progenitors of endothelial cells (31), and even increase the numbers of circulating endothelial progenitor cells (32). This may lead to an increase in bone formation, since vascular invasion is a prerequisite for calcification during endochondral bone formation (33).

Perhaps the most pertinent to HIV and this proposal, is the fact that statins may also affect bone formation indirectly by inhibiting inflammation. A recent study showed that statins antagonize TNF-alpha inhibition of BMP-2-induced osteoblast differentiation (34). Statins inhibit the RANKL-induced NF-kappaB activation pathway that leads to suppression of osteoclastogenesis induced by RANKL (35). In addition, statins may enhance osteoblastic differentiation and production of OPG by human osteoblasts (36). Although it seems less likely that the effects on inflammation have an important effect on bone formation in normal subjects, in states with heightened chronic inflammation, like HIV, this effect may be prominent.

In the general population, several human studies have assessed the role of statins on BMD and fractures. In a cross-sectional population study, an association was found between BMD and statin use in post-menopausal women (37).BMD of the spine and hip were approximately 7–8% higher in women taking statins than in controls of similar age, height, weight, years since menopause, and use of hormone replacement therapy. A case-control study using the health-maintenance records from 928 women aged 60 years or over with a fracture of the hip, humerus, distal tibia, wrist, or vertebrae showed a lower risk of fracture (odds ratio [OR] 0.48 [95% confidence interval [CI] 0.27–0.83]) in those who had taken statins for at least one year than in 2747 controls with no fracture (38). This was maintained after excluding individuals taking osteoporosis treatments and after adjusting for age and number of hospital admissions and score for chronic disease. A nested case-control study using a UK-based general practice research database has also shown that current use of statins was associated with a reduced risk of fracture (OR 0.55 [95% CI 0.44–0.69]) (39). The study included 28,340 men and women aged > 50 years taking lipid-lowering drugs, 13,271 with hyperlipidemia not taking lipid-lowering drugs, 50,000 randomly selected individuals without hyperlipidemia, and 3940 individuals with a previous bone fracture. Results were controlled for body mass index, smoking, and use of corticosteroids or estrogen. In a case-control study of 6110 individuals aged > 65 years, the use of statins in the previous 180 days (OR 0.5 [95% CI 0.33–0.76]) or previous 3 years (OR 0.57 [95% CI 0.40–0.82]) was associated with a reduction in hip fracture (40). This reduction persisted even after adjustment for race, estrogen use, and a number of chronic diseases. The possibility that these effects were via reducing cholesterol levels

seems unlikely, because all the above studies showed no effect from non-statin cholesterol-lowering drugs.

In contrast, few studies have failed to demonstrate an association between statin use and risk of fracture. A randomized trial looked at the frequency of fractures occurring in a large group of patients with ischaemic heart disease treated with pravastatin in the LIPID study (41). There was no difference in fractures occurring in the pravastatin group (n = 107) as compared with the placebo group (n = 101) (OR 1.05 [95% CI 0.80–1.37]). Another study used the same General Practice database as Meier et al but different analytic methods and time periods and a slightly different subsample (41, 42). They found no association between use of statin and risk of fracture in 81,880 individuals sustaining a fracture of the vertebrae, clavicle, humerus, radius/ulna, carpus, hip, ankle, or foot after adjusted for smoking, medications, and illnesses associated with risk of fracture. However, the results suggested a modest protection for hip fracture.

In addition to these cohorts/trials, few human studies have investigated the effect of statins on bone markers. Three months of statins showed significant decreases in bone resorption markers, with a trend towards a decrease in bone formation markers, in two separate studies of patients with hypercholesterolemia. (43,44). Another study found that statins also cause a significant decrease in the ratio of C-telopeptide to osteocalcin, an indicator of bone remodeling, but only in older subjects, suggesting an exclusive beneficial effects on bone turnover in older individuals (45).

3.3. Statins, specifically rosuvastatin in HIV

Rosuvastatin is a highly potent 3-hydroxy3 methylglutaryl coenzyme A (HMG-CoA) inhibitor and is not metabolized by the Cytochrome P3A4 (CYP3A4) enzyme system, which is frequently inhibited by certain ART. The lack of requirement of CYP3A4 metabolism for rosuvastatin makes it an attractive lipid-lowering drug to treat the dyslipidemia associated with ART. To date only two studies have examined the effect of rosuvastatin for treatment of ART-related dyslipidemia, and none assessed changes in endothelial function or atherogenesis. One of the studies (n=70) found a 25% reduction in total cholesterol, a 21% decrease in triglycerides, and a 31% reduction in LDL-cholesterol (46). Interestingly, in this study, the 10 mg subgroup (n = 45) showed a greater improvement across cholesterol, LDL-cholesterol and TG parameters relative to the 20 mg subgroup (n = 23). The second study treated 17 patients with hypercholesterolaemia with 10 mg a day rosuvastatin for 24 weeks. Significant reductions were seen in total cholesterol of 22% and in triglyceride levels of 30% ($P < 0.01$). (47). In both studies, rosuvastatin was found to be safe even when the vast majority of subjects in these studies were on PI-based ART. In addition, one study compared the efficacy of rosuvastatin vs. pravastatin vs. atorvastatin in HIV-infected subjects with hypercholesterolemia (48). The mean decrease in total cholesterol was significantly greater with rosuvastatin (25.2%) than with pravastatin (17.6%; p=0.01) and atorvastatin (19.8%; p=0.03). In this study, 12 months of all 3 statins studied showed a favorable tolerability profile, and no asdverse effect on plasma HIV-1 RNA.

One of the postulated mechanisms of pro-atherogenic effects of ART, specifically PIs is the promotion of atherosclerotic lesion formation by an increase in CD36-dependent cholesteryl ester accumulation in macrophages. Additionally, hypercholesterolaemia promotes a CD36-dependent and endothelial nitric oxide synthase mediated endothelial dysfunction. Thus endothelial dysfunction is expected in subjects on ART as has been demonstrated (15-18). To date, only 2 small studies have studied the effects of statins (both used pravastatin) on endothelial function in HIV. A small randomized placebo-controlled trial (n=29) in ART-treated HIV-infected subjects with hypercholesterolemia

revealed that 8 weeks of 40 mg pravastatin significantly improved endothelial function, as assessed by FMD (49).. On multivariate analysis, change in LDL-cholesterol was inversely related to change in FMD. The second small study (n=20) found a strong trend towards improvement of endothelial function after 40 mg of pravastatin (50). Of note in this latter study, pravastatin resulted in a significant decrease in atherogenic lipoproteins, particularly those most associated with future coronary events; a 21% reduction in LDL particles (P =0.03), a 27% reduction in small LDL (p =0.100), and a 45% reduction in small VLDL (P =0.02).

Importantly, none of these pravastatin studies assessed carotid IMT, markers of inflammation or oxidative stress after the intervention. Thus our study is novel, using a different more potent statin, and will answer important research questions; it will be the first comprehensive and well powered trial that will assess the effect of statins in modulating cardiovascular risk and endothelial function in subjects regardless of lipid levels. Also we will take advantage of this well designed intervention to assess pathogenesis aspects of cardiovascular disease that have not been previously investigated in HIV.

Evaluation of ventricular function

HIV infection is associated with myocardial dysfunction, although dilated cardiomyopathy is typically seen only in advanced stages of AIDS. HIV-infected patients are now living longer in the modern era of antiretroviral therapy, and appear to be at increased risk for a variety of cardiovascular diseases including coronary artery disease and heart failure (55). Although impaired ejection fraction is less common, HIV-infected patients on antiretroviral therapy have a higher prevalence of diastolic dysfunction and left ventricular hypertrophy compared to HIV-uninfected controls (56, 57). Whether subclinical abnormalities of systolic function measured by speckle tracking strain echocardiography are prevalent in an older HIV-infected population is unknown. In other diseases characterized by chronic inflammation such as rheumatoid arthritis(58) and systemic lupus erythematosis (59),abnormalities of echo derived systolic strain are prevalent despite normal ejection fraction.

In this hypothesis generating pilot study, we aim to explore whether HIV-infected patients have lower global longitudinal strain compared to a HIV uninfected normative reference population. In addition, we will explore the relationship between global and regional LV strains and strain rates and biomakers of inflammation, immune activation, coronary artery calcium score, epicardial fat volumes, carotid artery distensibility, carotid intima-media thickness, and flow-mediated dilation of the brachial artery.

Evaluation of vascular inflammation: PET imaging substudy

Atherosclerosis is an inflammatory disease (61) and HIV infection may accelerate the development of atherosclerosis by increasing systemic and/or local vascular inflammation (62). 18-Fluorine-2-deoxy-D-glucose positron emission tomography (FDG-PET) is a technology that is used to measure inflammation within the walls of large arteries such as the aorta or carotid. It appears that HIV infection is associated with higher levels of aortic inflammation by PET when a CT scan is used for tissue localization and attenuation correction (63); however, whether PET with MRI and gadolinium enhancement sequences might be able to better characterize the nature and extent of inflammation in patients with chronic treated HIV infection is unknown. Also no study to date has assessed the relationship between vascular inflammation (by PET) and markers of immune activation and systemic inflammation. Thus in this pilot substudy of chronic HIV-infected subjects on ART we aim to explore whether systemic markers of inflammation and immune activation obtained as part of the 96 week evaluation are associated with aortic and carotid inflammation in a subgroup of subjects enrolled in the PET imaging substudy.

New CT Analysis:

Background and aims:

To further characterize the nature of calcified coronary plaque in patients with treated HIV infection and the changes with statin therapy over time, we plan to reanalyze raw cardiac CT images from the SATURN trial at 0, 48, and 96 weeks. We will perform advanced feature detection upon serially acquired coronary calcium CT and quantify changes in these features. We will then compare these features to soluble biomarkers obtained at the same time points.

Aim 1. Develop tools for measuring subtle changes in coronary calcium. We will register serial coronary calcium CT image volumes. We will apply visualization techniques and change detection techniques commonly applied to other applications in medical imaging (tumors and brain dementia) to determine subtle progression/regression patterns. We will segment coronary calcifications and apply methods for examining extract a large number of features, including standard Agatston, mass, and volume calcium scores, as well as new assessments including: length; circumferential coverage; peak intensity; average intensity; average, maximum, standard deviation of thickness, etc.

Aim 2. Quantify the progression of coronary calcium over time using CT calcium score in HIV infected patients on statin therapy as compared to control. We will use measurements from Aim 1 and appropriate statistical or machine learning approaches to analyze data.

Aim 3. Determine if changes in soluble biomarkers correspond with changes in coronary artery calcium. We will measure serum hs-CRP, IL-6, and TNF-α. We will also measure the change in proportion of inflammatory monocyte activation as well as the change in proportion of activated T cells. This will be performed in those who have undergone CT calcium scoring and measurement of inflammatory markers measured at the same time points.

4. STUDY DESIGN AND APPROACH

This study is a phase II randomized, double-blind, placebo-controlled study of rosuvastatin. HIV-1 infected subjects of 18 years of age and older, HIV-1 RNA ≤ 1,000 copies/mL, LDL-cholesterol ≤130 mg/dL, triglyceride levels ≤500 mg/dL, evidence of heightened immune activation (hsCRP ≥2 mg/L or CD8+ HLA-DR+ CD38+ ≥ 19%), and who have at least one additional risk factor for CVD (such as hypertension, low HDL-C (less than 50), smoking, male sex and women ≥60 years of age, or a family history of premature coronary heart disease), who are currently receiving stable ARV therapy for at least 12 weeks will be enrolled in these studies. The cumulative duration of ARV before study entry should be at least 6 months. Subjects should have no intention to change ART, or modify their diet during the study period.

The duration of the study will be 96 weeks. Patients will be randomized equally to one of 2 treatment arms.

1) Rosuvastatin 10 mg once daily
2) Matching placebo once daily

Patients will be stratified by 1) by receiving PI- or no PI- containing regimen at study entry and 2) by BMD (lumbar spine or hip t-score < -1 vs. both lumbar and hip t-score ≥-1)
3) CT calcium score (Agatston score 0-99 vs ≥ 100).

Study drugs will be provided free of charge for study participants. Antiretroviral therapy will not be provided by the study. The decision for ARV changes or discontinuation will be left to the primary care provider, but these changes will be documented in the study chart and will be taken into account during the analyses. The duration of the study will be 96 weeks, as this was shown to be appropriate in similar trials for observing changes in carotid IMT, flow-mediated dilation,

endothelial and inflammatory markers, bone metabolism, as well as changes in glucose metabolism and lipids.

5. SELECTION AND ENROLLMENT OF SUBJECTS

5.1. INCLUSION CRITERIA

5.1.1. HIV-1 infection as documented by any licensed ELISA test kit and confirmed by Western blot at any time prior to study entry. HIV-1 culture, HIV-1 antigen, plasma HIV-1 RNA, or a second antibody test by a method other than ELISA is acceptable as an alternative confirmatory test

5.1.2. Age ≥18 years

5.1.3. Receiving a stable antiretroviral regimen for at least the last 12 weeks prior to study entry

5.1.4. Cumulative duration of antiretrovirals for at least 6 months at study entry

5.1.5. Provides written informed consent and is capable of reading and comprehending the informed consent

5.1.6. All women of child-bearing potential (WOCBP) must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of □HCG) within 72 hours prior to start of study medication. WOCBP is defined as any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy), who is not postmenopausal (defined as amenorrhea □12 consecutive months), or is on hormone replacement therapy (HRT) with documented plasma follicle-stimulating hormone level □35mLU/mL. Women who are using oral, implanted, or injectable contraceptive hormones or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where partner is sterile (e.g., vasectomy), should be considered to be of child bearing potential.

5.1.7. Female subjects who are not of reproductive potential (have reached menopause or undergone hysterectomy, bilateral oophorectomy or tubal ligation) or whose male partner has undergone successful vasectomy with resulting azoospermia or has azoospermia for any other reason, are eligible without requiring the use of contraception. Acceptable documentation of menopause, sterilization, and azoospermia is patient reported history.

5.1.8. All subjects must not participate in a conception process (e.g. active attempt to become pregnant or to impregnate, sperm donation, in vitro fertilization), and if participating in sexual activity that could lead to pregnancy, the female subject/male partner must use condoms (male or female) in addition to one of the following forms of contraception while on study: either a spermicidal agent, diaphragm, cervical cap, IUD, or hormonal-based contraception.

5.1.9. Karnofsky performance score □ 80

5.1.10. Have no plans to alter antiretroviral therapy, diet or exercise or initiate structured/strategic antiretroviral treatment interruptions.

5.1.11. Documentation of at least 2 consecutive HIV-1 RNA levels of ≤1,000

copies/mL using the UltraSensitive Roche Amplicor assay (1.5 assay) for at least 6 months prior to study entry

5.1.12. Fasting LDL-cholesterol ≤130 mg/dl

5.1.13 Fasting triglycerides ≤500 mg/dL

5.1.14. Evidence of immune activation (hsCRP ≥ 2 mg/L or CD8+ HLA-DR+ CD38+ ≥ 19%)

5.1.15. Other CVD risk factor

Inclusion Criteria for Optional ECHO for participants currently enrolled in the rosuvastatin trial

- Willing to undergo an additional test at the week 48 or week 96 visit
- ≥44 years of age
- One or more of these risk factors (HTN, low HDL (<40mg/dL for men or <50mg/dL for women), DM, smoking, family history of coronary heart disease)

The first 50 participants who meet these inclusion criteria and consent to participate will be enrolled in this sub-study

Inclusion Criteria for Optional PET/CT and PET/MR for participants currently enrolled in the rosuvastatin trial

- Willing to return within 30 days of the week 96 visit for an additional test
- One or more of these risk factors (HTN, low HDL (<40mg/dL for men or <50mg/dL for women), smoking, family history of coronary heart disease)
- GFR > 75
- ABSCENCE of metal inside the body (e.g. heart pacemaker, artificial heart valves, metal implants such as metal ear implants, bullet pieces, chemotherapy or insulin pumps or any other metal such as metal clips or rings)
- Glucose of <200 (non fasting) or <126 fasting within 30 days of the PET evaluation
- No history of kidney disease or liver or kidney transplantation

The first 20 participants who meet these inclusion criteria and consent to participate will be enrolled in this sub-study.

5.2. EXCLUSION CRITERIA

5.2.1. History of myocardial infarction, unstable ischemic heart disease, stroke, or coronary revascularization procedure

5.2.2. Women who are pregnant or breastfeeding.

5.2.3. Women with a positive pregnancy test on enrollment or prior to study drug administration.

5.2.4. A clinically important illness within 14 days prior to study entry not explicitly excluded by the protocol, a physical or psychiatric disability, or a laboratory abnormality that might place the subject at increased risk by being exposed to the medications in this study or which might confound the interpretation of this investigation.

5.2.5. Any active or chronic uncontrolled inflammatory condition

5.2.6. Diarrhea or vomiting of Grade ≥ 2 within 14 days prior to study entry

5.2.7. An active AIDS-defining opportunistic infection or disease (for the purpose of this study, a CD4 count □200 cells/mm^3 in the absence of any other AIDS-defining indicator condition is not considered an AIDS-defining event).

5.2.8. Inability to communicate effectively with study personnel.

5.2.9. Current alcohol or recreational drug use which in the investigator's opinion interferes with the subject's ability to comply with dosing schedule and protocol evaluations.

5.2.10. Any change in the last 24 weeks in therapy with prescription omega-3 fatty acids (>2 gram/day of lovaza), fibrates, or prescription niacin. Therapy with OTC omega 3 fatty acids or with ≤ 2 g lovaza is allowed. Any statin therapy for longer than 14 days received in the last 24 weeks is exclusionary

5.2.11. Use of the systemic cancer chemotherapy, or immumodulating agents, such as Etanercept, Infliximab, or other cytokines modulators within 60 days prior to study entry.

5.2.13. Uncontrolled hypothyroidism or hyperthyroidism

5.2.14. Uncontrolled diabetes (HbA1C >8.5%)

5.2.15. Any change in lipid modification pharmacotherapy or hypolipemics or hypoglycemic therapy in the last 24 weeks prior to study entry

5.2.16. Known underlying myositis or muscle disease

5.2.17. Use of anabolic agents, growth hormone, growth hormone releasing factor, or any other anabolic agents, except for stable replacement testosterone

5.2.18. Male subjects on testosterone replacement therapy must have been on stable therapy for at least 3 months prior to study entry and must be willing to continue stable therapy for the duration of the study. Stable therapy is defined as continuous active therapy with no alterations of any kind, including dose, dose frequency, or formulation of any agent.

The following forms of testosterone replacement are allowed:

- Injectable testosterone enanthate/cypionate/propionate in doses of 400mg/month or less.

- Testosterone patches:
  Testoderm TTS (Testosterone Transdermal System) ≤5mg/day patch

  Testoderm ≤4mg/day patch

  Testoderm with Adhesive ≤ 6mg/day patch

  Androderm ≤2.5mg/day patch and ≤5mg/day patch,

- Testosterone gel (Androgel) ≤7.5 gm per day.

5.2.19. Use of bisphosphonates or other bone therapies

5.2.20. Any of the following laboratory findings obtained within 14 days prior to the screening evaluation including the following:

- AST and/or ALT >2.5 x ULN
- Hemoglobin 9.0 g/dL
- Non exercise Creatine kinase (CK) > 3 X ULN
- Calculated creatinine clearance <50 mL/min as estimated by the Cockcroft-Gault equation:

  For men, (140 – age in years) x (body weight in kg) ÷ (serum creatinine in mg/dL x 72) = CrCl (mL/min)*
  For women, multiply the result by 0.85 = CrCl (mL/min)
  NOTE: We will use the ACTG calculator system available at:
  http://www.fstrf.org/ACTG/ccc.html

6. SCHEDULE OF EVENTS

	Screening	Entry*	Week 6	Week 12	Week 24*	Week 36	Week 48*	Week 72	Week 96*
Written consent form	X								
Inclusion/exclusion	X								
Family history of CVD/diabetes/HTN/hip fracture	X						X		X
Targeted physical exam/weight/blood pressure	X	X	X	X	X	X	X	X	X
Compliance assessment/safety monitoring			X	X	X	X	X	X	X
Smoking, elicit drugs and alcohol status		X			X		X		X
Dietary logs and Activity Status		X			X		X		X
Height		X					X		X
Waist circumference, WH ratio		X			X		X		X
Hematology/chemistries	X	X	X	X	X	X	X	X	X
Urine -HCG (Women only)	X	X			X		X		X
CD4+/CD8+ cell count**		X			X		X		X
Plasma HIV-1 RNA**	X	X			X		X		X
Fasting lipids,	X				X		X		X
Calculation HOMA-IR, QUICKI,		X			X		X		X
oral glucose challenge		X			X		X		X
Hb1AC	X				X		X		X
Inflammation markers, KLF2/4 (Batched)		X			X		X		X
HSCRP and CD8+ HLA-DR+ CD38+	X								
Oxidative markers (Batched)		X			X		X		X
CT chest—coronary calcium and pericardial fat	X						X		X

Carotid IMT		X					X		X
Brachial artery FMD		X			X		X		
Whole body DEXA/Bone DEXA		X					X		X
ECHO****							X		X
PET/CT; PET/MR*****									X
Bone markers		X			X		X		X

(Batched)									
Stored fasting plasma, serum, PBMCs, whole blood and urine***		X			X		X		X

*Screening, Entry, Week 24, week 48 and 96 visits will be done in fasting state (after at least 8 hours fasting). We will also require a 12-hour smoking and caffeine free period before each of the brachial reactivity measurements.

**CD4+/CD8+ cell count/Plasma HIV-1 RNA will be obtained from clinical chart as it will be done as part of routine care.

*** Plasma, whole blood, PBMCs, urine and serum will be stored in the local laboratory in a -70 degrees freezer (-150 for PBMCs) for potential future metabolic, cardiovascular, oxidative markers, inflammation markers, vitamin D and bone metabolism markers, EBV viral load and immune activation/senesence. Urine will be used for possible oxidative and bone markers. Some of this stored blood may be used for measurements of antiretroviral concentrations.If subjects consent to genetic testing, some of the material may be used for future genetic testing, looking at genetic predisposition to cardiovascular, bone and vitamin D metabolism.

Subjects who prematurely withdraw from the study will have the same evaluations, as outlined for week 96

****ECHO will be an optional procedure for the first 50 participants who qualify. The procedure will take place at week 48 or week 96 depending on the time point the participant is at.

*****PET/CT, PET/MR will be an optional procedure for participants who qualify and willing to return within 30 days of the week 96 visit for procedures.

7. TIMING OF EVALUATIONS

Screening

All screening evaluations to determine eligibility must be completed within 30 days prior to study entry.

Entry

Evaluations must occur at least 24 hours and within 30 days after screening evaluations. Subject must begin treatment within 96 hours after randomization. Results of the pregnancy test must be known prior to initiation of study treatment.

No further evaluations are required for subjects who are randomized and do not have study medications dispensed. These subjects will be replaced.

On-Study Evaluations

The schedule is followed after randomization. Study visits must be scheduled on the weeks indicated in the schedule of events ±14 days. For entry, Week 24, 48 and 96, a ±28 days window will be accepted.

Premature study discontinuation

Subjects who stop study treatment or discontinue antiretroviral therapy will

undergo the premature study discontinuation evaluations as outlined in the Schedule of events under Week 48 visit.

Pregnancy

Rosuvastatin is not safe in pregnant women. Women who become pregnant while on study drugs will be immediately discontinued from study drugs and will not be followed any further.

8.0 DETAILED STUDY PROCEDURES BY VISITS

Screening:

- Before they enter, patients will be asked to visit the clinic in a fasting state at least once to be screened and ensure that they meet the requirements for entry into the study. Screening may take place on the same day as a normally scheduled clinic appointment. Before any tests can be obtained as part of this study, patients would have to decide whether or not they would like to participate in this study. If they choose to enroll, this informed consent form will be signed. A targeted physical examination will be performed and recent blood work will be reviewed to be sure that they meet the inclusion criteria. The HIV-1 RNA, and HIV testing to confirm HIV status will be obtained from the clinical chart as these are a part of routine care. For woman of reproductive potential, a urine sample will be taken for pregnancy test. . A CT scan of the chest (without contrast) will be performed for coronary calcium score and pericardial fat. This test takes about 15-25 minutes. Results will not be available until the end of study. If a patient should be re-screened and had a CT scan within the previous 90 days for this study, the CT scan will not be repeated.

Study Entry/On-study evaluations

For entry visit, this will be fasting, as defined by no food or drink, except for plain water and medications for at least 8 hours. The following procedures will be done at entry.

- A medical history and a targeted physical exam. Patients will fill out a dietary and physical activity questionnaire.

- Blood will be obtained for markers of inflammation and endothelial activation, inflammation glucose and insulin, a complete blood count, chemistries and hematology.HIV-1 RNA, and CD4 count will be obtained from the clinical chart as these are part of routine care.

- Some of the plasma and serum will be stored for potential future testing such as additional tests for inflammation, cardiovascular markers, endothelial function markers, oxidative markers, EB virus viral load, and bone metabolism. These blood samples will be batched and the tests will be done at the end of the study and are part of this study. Genetic tests may be done but are optional and obtain only if subjects allow genetic testing in the written consent form.

- Intima-media thickness (IMT) test, FMD, will be performed. In addition, a DEXA scan will be done for body composition and bone density measurement.

- Patients will be given an oral glucose challenge test and have blood drawn two (2) hours later to measure their blood glucose and insulin levels.

- After signing the consent, patients will be randomized to one of two groups. The first group (Group 1) will be the group that receives the study medication rosuvastatin. The second group (Group 2) will receive a placebo (inactive form of rosuvastatin).

- At this visit patients will be given the study medication (either active rosuvastatin or the

placebo, depending on the randomization). They will also be given instructions for how many pills they should take each day.

The follow up study visits will be 6, 12, 24, 36, 48 and 72 weeks after the first visit. Patients will be asked to bring all of the pills that they have left over to these visits. They only need to come fasting for the visits at week 24 and 48.

- At each of these visits, patients will be asked about symptoms or any change in health status. A short targeted physical exam will be done.

- Blood will be collected for a complete blood count, chemistries, and hematology.

- Next, a pill count will be done of the remaining pills that patients have to ensure adherence.

- For study visit at week 24 and 48, additional testing will be done (similar to baseline visit) to check for inflammation markers, oxidative markers, and insulin. HIV-1 RNA, and CD4 count will be obtained from the clinical chart. Lipids will also be drawn at these visits. In addition on week 24 and 48, patients will be given an oral glucose challenge test and have blood drawn two (2) hours later to measure their blood glucose and insulin levels. Also a urine pregnancy test will be done if applicable. In addition, for study weeks 24 and 48, some of the blood will be stored for potential future testing such as additional tests for inflammation, oxidative stress, cardiovascular markers, bone metabolism and EB virus viral loads. Patients will also be asked about diet and level of physical activity. Also the flow mediated dilation test will be done again at weeks 24 and 48 in a similar way than at entry. Also the carotid IMT, CT scan, and DEXA scan will be done at week 48. At these follow up visits, a medical history and targeted physical exam will be done.

The final visit will be 96 weeks after the initial study visit. At this visit, patients will be instructed to come fasting as defined for the entry, week 24, and 48 visits. They will also be instructed to bring all of the pills that they have left. At this visit all of the procedures that occurred at the first visit and the Week 48 visit will be repeated. Also a final pill count will be done.

- At this visit a medical history and targeted physical exam will be done.

- Blood will be drawn for markers of inflammation, oxidative markers, glucose, insulin, lipids, tests for bone markers, a complete blood count, chemistries, haematology. HIV-1 RNA, and CD4 count will be obtained from the clinical chart. Patients will be given an oral glucose challenge test and have blood drawn two (2) hours later to measure their blood glucose and insulin levels. In addition, women will have a urine pregnancy test.

- The IMT test, CT scan for calcium scoring and pericardial fat (pericardial fat will be defined as any fat tissue inside the pericardial sac), and the DEXA scan will be performed again as previously described.

- Some of the blood and urine will be stored for potential future testing such as additional tests for inflammation, cardiovascular markers, oxidative markers, bone and vitamin D metabolism, and EB virus viral load as needed by the study. Also some of the blood may be used to measure levels of antiretrovirals.

9. EXPERIMENTAL DESIGN/ METHODS

9.1. Targeted Physical Examination:

A targeted physical examination is to include vital signs (temperature, pulse, respiration rate, and blood pressure) and is to be driven by any previously identified or new signs or symptoms including diagnoses that the subject has experienced within 30 days prior to screening or since the last visit. The exam should also include a description of any abnormal fat deposition, striae, bruising, facial plethora, and bone pain within 30 days prior to study entry or since the last visit.

9.2. Weight

Collect weight at all study visits

9.3. Height

Collect height at entry, Week 48 and 96 weeks.

9.4. Concomitant Medications

All concomitant medications taken since the last report will be recorded in the source documents and the CRFs. While the use of the following medications during the study will not result in study discontinuation, information regarding these medications will be recorded in the CRF and will include start and stop dates, and the indication (clinical diagnosis) that led to the use of these medications.

- Niacin
- Ezetimibe
- Fibrates
- Fish oil
- Any antihypertensives or treatment for heart disease
- Estrogens
- Steroids
- Glitazones, metformin or other diabetes treatment
- Anabolic agents (including testosterone)
- Corticosteroids
- Vitamin supplements, Coenzyme Q, N acetyl cystein, L-acetyl carnitine, uridine supplements. All vitamin D doeses and changes will be collected in details in the CRFs.

<u>Antiretroviral Medications</u>

Antiretroviral medications will not be provided by the study. Subjects will continue antiretroviral medications and any decisions regarding antiretroviral therapy will be made by the subject's primary care provider. All modifications to antiretroviral medications will be noted, including dose and start and stop dates on the CRFs.

9.5. Adherence assessment

At each visit, the amount of dispensed and returned pills will be counted and recorded in the CRFs.

9.6. Dietary logs

All participants' dietary logs will be collected at study entry, week 24,48, and week 96 to assess if significant dietary changes occurred during the study and whether these changes influenced any of the study endpoints. Reported dietary intake will be analyzed at the end of the study for energy intake using the Nutrition Data System for Research (NDS-R) software version 2005

9.7. Physical Activity Status: will be assessed by standardized questionnaire adopted from the ACTG

9.8. Anthropometric measurements: Baseline (study entry), 24-, 48- and 96- week weight, height, and waist and hip circumferences will be measured by an experienced registered dietitian or a trained registered nurse. Measurements will be taken in a fasting state, and will be done as follow (adopted from ACTG guidelines). - The subject will be dressed in underwear, socks, and a hospital gown; all outer clothing will be removed. All measurements will be made in triplicate.

Waist Circumferences

- The subject should be standing erect but relaxed, and should not try to hold in the stomach.
- All measurements will be made after subject has exhaled
- The usual method is to measure the smallest circumference around the waist. However, this measurement is not sufficient in individuals with increased abdominal girth. Therefore, we will obtain three different circumferences; in each case, the measuring tape should be parallel to the floor during the measurement and measurements repeated in triplicate
- Minimal waist (conventional): Viewing the subject from the front or rear, identify the smallest width of the waist; measure circumference at that point.
- Umbilicus waist: Measure circumference at the level of the navel.
- Midwaist: Locate the upper border of the right ilium and measure the waist circumference at this level. The tape measure should be parallel to the floor.

Hip Circumference

- The subject should be standing erect but relaxed, and should not try to hold in the stomach.
- Viewing the subject from the side, visually identify the widest width of the hip. The hospital gown may be held to conform to the subject's contour; the widest point is generally where there is maximal protuberance of the buttocks.
- Measure circumference at that point, making sure the measuring tape is exactly parallel to the floor.
- Record the result in cm to the nearest millimeter. The procedure will be repeated twice, for a total of 3 measurements.

9.9. Hematology and Chemistries

Hemoglobin, hematocrit, red blood cells (RBC), white blood cell count (WBC), differential WBC, absolute neutrophil count (ANC), and platelets required.

Creatine kinase, glucose, electrolytes [sodium, potassium, chloride, phosphate, bicarbonate], calcium, magnesium, phosphate, lactate dehydrogenase (LDH), creatinine, uric acid, total protein, albumin, total and indirect bilirubin, AST (SGOT), ALT (SGPT), alkaline phosphatase, and □-glutamyl transaminase (GGT).

9.10 HIV-antibody test

HIV-1 infection should be documented by any licensed ELISA test kit and confirmed by Western blot at any time prior to study entry. HIV-1 culture, HIV-1 antigen, plasma HIV-1 RNA, or a second antibody test by a method other than ELISA is acceptable as an alternative confirmatory test.

9.11. HIV-1 RNA

Plasma HIV-1 RNA and CD4 cell counts will be performed as part of routine clinical care.

9.12. Immune activation

Immune activation will be obtained at baseline, week 48 and week 96. An HIV-1-focused measurement of immune activation and immune senescence will also be performed on peripheral blood CD8+ T-cells, quantifying the presence of the CD38, as an activation marker and the absence of CD28, a widely used senescence marker over the course of the study.

9.13. Pregnancy test

For women with reproductive potential: Serum or urine □-HCG (urine test must have a sensitivity of 25-50 mIU/mL) must be performed. Testing must be repeated whenever pregnancy is suspected, in addition to the regularly scheduled evaluations. All pregnancy test results must be recorded on the CRF. The subject must NOT receive study drugs until the result of the entry pregnancy test is known to be negative.

9.14. Fasting lipoproteins and insulin/glucose

If subjects come to the appointment and are not fasting, they will need to come back for these evaluations within 96 hours.

Total cholesterol, HDL-cholesterol, LDL-cholesterol (calculated direct LDL, triglycerides, insulin and glucose will be performed in real time at the local laboratory.
Oral glucose challenge: Insulin and glucose will be measured at 0 and 120 minutes after a 75g oral glucose challenge

9.15. Assessment of metabolic syndrome:

The prevalence of the metabolic syndrome will be assessed before and after the interventions by using the guidelines initially established by the 2001 National Cholesterol Education Program Adult Treatment Panel (ATP III). The modified version of the guidelines was issued by a statement of the American Heart Association and the National Heart, Lung and Blood Institute, published in Circulation in 2005. Specifically in our study, the metabolic syndrome will be defined as having three or more of the following characteristics: waist circumference ≥88 cm, fasting triglyceride levels ≥150 mg/dl (or hypolipemic agents known to decrease triglycerides), HDL cholesterol <50 mg/dl, fasting glucose □ 100 mg/dl (or on hypoglycemic agent) and blood pressure ≥130/85mm Hg or currently on antihypertensives with history of hypertension. The prevalence of the metabolic syndrome in HIV-infected subjects has been as high as or even higher than in HIV uninfected population, ranging from 25-45%. These studies used unselected individuals, who were not specifically recruited because of known cardiovascular risk factors. Thus, we expect a high prevalence in our study population

9.16. Inflammatory markers

We will measure plasma hsCRP, IL-6, TNF-□, soluble TNFRI and II, OPG and RANKL as markers of chronic inflammation. The assessment of these markers before and after the intervention is novel and has not been performed to date in HIV-infected patients. We will measure hsCRP by particle enhanced immunonepholometric assay on a BNII nephelometer (Siemens), and IL-6, soluble

TNFR I and II, and sVCAM-1 by ELISA (R&D Systems, Inc., Minneapolis, MN).

9.17. F2-Isoprostanes and oxidized LDL

Quantification of F_2-isoprostanes is widely considered as the most accurate method to measure oxidant stress in vivo (51, 52). Measurements will be performed on urine which after collection, had been immediately stored at -70°C until testing. F_2- isoprostanes are quantified using Mass Spectroscopy analysis.after Sep-Pak and TLC purification as pentafluorobenzyl ester, trimethylsilyl ether derivatives utilizing stable isotope dilution techniques using deuterated 15-F2t-IsoP (Cayman Chemical, Ann Arbor, MI) as an internal standard. The precision of the assay is 4% and the accuracy 95% and interassay variability is less than 8% (53). Serum oxidized-LDL levels will be measured using an ELISA assay (Mercodia, Uppsala, Sweden). Serum oxidized- LDL levels will be measured using an ELISA assay (Mercodia, Uppsala, Sweden). Oxidized LDL concentrations will be expressed as an absolute measurement and as a ratio of oxidized LDL/total LDL, a relative measure of lipid peroxidation.

9.18. Kruppel-like factors

PBMCs will be isolated using standard techniques. RNA will be stabilized by addition of RNAprotect (Qiagen) to the cell prep. RNA will be isolated, converted to cDNA and assessed using probe-based quantitative PCR. Data will be expressed as fold change from control (DDCt).

9.19. Carotid IMT:

Carotid intima medial thickness will be used as an objective surrogate for the presence and extent of coronary artery disease. As we have previously done, all carotid IMT will be done by the same experienced sonographer, and will be read by an experienced vascular medicine physician. Both the sonographer and the physician will be blinded to clinical history and study arms.

Summary of the carotid IMT protocol: Using a standardized protocol and digital image acquisition IMT will be scored as per the protocol of Stein et al (54). All studies will be performed with a Phillips iU22 ultrasound system and L9-3 MHz linear array transducer (Phillips Medical Systems, Andover, MA). Patients will be positioned supine with their head slightly hyperextended and neck rotated away from the ultrasound probe. The bilateral common carotid arteries (CCA), internal carotid arteries (ICA) and external carotid arteries (ECA) will be imaged in B-mode in the transverse and longitudinal views. Pulsed wave Doppler will be obtained of the origin and proximal ICA and ECA bilaterally. If any plaque, velocity shift or stenosis is identified complete assessment will be performed. Using a perpendicular imaging plane and Meier's arc for probe positioning, longitudinal, R-wave gated cine-loop and still frame images of the distal 1 cm of the CCA far wall (free of plaque) will be acquired. Three measurements of the IMT will be obtained of each distal common carotid artery using 3 different imaging planes. Carotid IMT thickness will be measured by leading-edge-to-leading edge technique using semiautomated border detection software. The average of three measurements at each site (right and left side) will be used as final measurement of IMT for that site. Results will be reported as mean-mean carotid IMT. Visible plaque (focal wall thickening > 50% of the adjacent vessel) will be defined, measured, and graded according to published protocols from the Cardiovascular Health Study.

9.20. Endothelial function by Flow mediated dilation

Assessment of brachial artery FMD is non-invasive, safe, and reproducible, and brachial artery FMD is strongly correlated with coronary artery FMD ($r=0.79$, $p<0.001$). Endothelial dysfunction predicts future atherosclerosis and is present

even before the appearance of arterial wall thickening. Pertinent to the use of FMD as a co-primary endpoint in this study, is the association between changes in FMD and CVD events with pharmacological interventions such as antihypertensive therapy and lipid-lowering therapy. As a functional marker of CVD that responds rapidly to interventions, the use of FMD is well-suited to study the effect of statins on CVD risk.

FMD of the Brachial Artery measures vasomotor function of the conduit brachial artery and has been used extensively by Dr Carman. We will use the lower arm occlusion method. Subjects will be placed in a supine position in a temperature-controlled room for 10 minutes prior to imaging. A blood pressure cuff is placed on the widest part of proximal right forearm approximately 1 cm distal to the antecubital fossa. The arm is extended 90° from the thorax and placed on an arm board with the elbow positioned downwards and the hand rotated so thumb pointed towards the ceiling. Using a high resolution (at least 7 MHz) linear array vascular ultrasound transducer, the brachial artery is located above the elbow and scanned in longitudinal sections with the focus zone set to the depth of the far wall. Time-gain compensation and overall gain settings are used to optimize images of the lumen/arterial wall interface. Extra-vascular landmarks in each subject are identified and labeled to assure that the imaged segment of the brachial artery is reproduced within and between studies. After recording baseline B-mode images of the artery and spectral Doppler images of flow, the forearm cuff is inflated to 250 mmHg for 5 minutes to induce reactive hyperemia. Immediately after deflation, spectral Doppler images are obtained to verify hyperemia. Repeat images of the brachial artery are obtained 60 and 90 seconds after cuff deflation. Blood pressure is measured in the contralateral arm at baseline and at time of cuff release. For each participant, the depth of field, gain, monitor intensity setting and other instrumentation settings from the baseline examination are used at the follow-up examination. Intra-and extra-arterial landmarks are used to assure fidelity of the imaging site over time, and the probe location and angle of insonation are standardized between images and studies. ECG-gating is used to identify end-diastole. A digital image of the initial scan is used to assist with matching of landmarks. Follow-up imaging uses the same ultrasound imager, transducer and other hardware used at the baseline examination. Follow-up imaging will be performed by the same sonographer. All imaging is performed digitally. We will not administer nitroglycerine since we re interested in endothelial-dependant assessments. Using this technique, the coefficient of variation for FMD measurement at our site is 1.8% which compares favorably with others. A mean improvement in FMD of at least 2-4% generally is required to detect a treatment effect in research studies. In statins studies, the treatment effect had ranged from 4-7% and this has been seen as early as after few days of therapy.

9.21. CT scan for Calcium scoring and pericardial fat assessment

The use of cardiac CT for CAC scoring to more accurately discriminate CVD risk has grown based upon the observation that CAC occurs almost exclusively in patients with atherosclerotic vascular disease and is not present in normal coronary arteries. A recent prospective cohort study showed that CAC scoring was a better predictor of subsequent events than was cIMT measurement (56). CAC changes significantly after as early as one year of statin therapy in HIV-uninfected populations (57,58), but changes in CAC after statins have not been assessed in HIV. Also for the first time in HIV, we will be able to compare baseline and changes in CAC and cIMT and assess the changes after statins in both modalities. Our site has had extensive experience with CAC scoring through its preventive cardiovascular medicine program, having screened 2,033 presumed intermediate risk patients with coronary calcium scoring between 10/2007 and 10/2008. The CAC will be quantified using an electron-beam CT scanner. For the entire study, the same experienced radiologist blinded to clinical data will read all CT scans using an interactive scoring system to calculate Agatston score. The intraobserver

agreement at our site is favorable (0.92).

Pericardial fat volume will be measured by a single reader using a semi-automatic segmentation technique on a dedicated offline workstation. Pericardial fat will be defined as any fat tissue inside the pericardial sac. Areas of pericardial fat will be traced manually and will be identified as pixels within a window of −195 to −45 Hounsfield units (HU) and a window center of −120 HU. 20 control cases will be compared to an experienced core laboratory (Dr. Chung Lieh-Hung, Taipei, Taiwan) using Bland-Altman analysis to generate coefficient of variation.

New CT analysis:
Image and serological data will be transferred to Professor Wilson's laboratory in the Department of Biomedical Engineering, Case Western Reserve University. Data will be kept in a secure server. All image data will be fully de-identified. Professor Wilson will be given a key which is sufficient to group patients into treated and untreated groups and which enables one to know the serial imaging studies for each person. Measurement data will then be recorded to an excel spreadsheet and uploaded directly into RedCap by study staff. Data analysis will be performed collaboratively with all study investigators including statisticians. Any clinically significant findings will be reports to the patient's PCP as determined by the PI.

9.22. Stored plasma, PBMC, serum, and urine, whole blood
Will be obtained in fasting state at entry, week 24, 48 and 96 and will be stored in the local laboratory in a -70 degrees or -150 freezers for future metabolic, oxidative, inflammation, and cardiovascular markers.

9.23. DEXA scans/bone assessment: Limb fat, lean body mass, trunk fat, and BMD of the lumbar spine and hip will be measured by dual-energy absorptiometry in the anteroposterior view (using the same GE Lunar Prodigy scanner for all subjects). For the hip, we will measure BMD at three different locations: total hip, femoral neck, and greater trochanter. Lumbar spine BMD will be measured from L1-L4. One experienced technician will scan the same hip of each patient and will use the same machine on the same individual throughout the study. DEXA will also measure simultaneously total body fat, leg and arm fat as well as trunk fat. We will also measure CTX as a marker of bone resorption and PINP as a marker of bone formation. The measurements of bone markers will be of particular importance in the event that BMD does not significantly change after the interventions, since bone markers are known to change much faster than BMD. In this case, we will use the stored samples to measure C-terminal telopeptide of type I collagen (CTx) as a marker of bone resorption, and PINP as a marker of bone formation. Several cytokines (plasma TNF-□, sTNF R-I, sTNF R-II, and IL-6) will be measured as part of the assessment of chronic inflammation and its relationship to cardiovascular and endothelial markers. These measurements will also be used to assess the relationship of baseline- and changes in BMD to those of these inflammatory markers. The pathogenesis of bone disease in HIV is unclear and could be linked at least in part to chronic inflammation. To date, longitudinal changes in OPG and RANKL and its relationship to TNF-□ and IL-6 have not yet been assessed in the setting of an intervention aimed to improve BMD in HIV.

Transthoracic echocardiogram with strain imaging

The patient will undergo a standard TTE with additional clips for strain analysis. The test will take place at the University Hospitals Heart & Vascular Institute (1800 Mather). The patient will lie on a bed for approximately 30-45 minutes while the ultrasound images are obtained with a probe placed on the breast bone and underneath the breast. There is no risk associated with the procedure, although there may be minor discomfort from pressure of the ultrasound probe.

Power: Compared to a normal reference population with a global longitudinal strain of -17±3% (mean±SD)(60), we will have 80% power to detect a mean difference of 1.36% with a two-sided alpha of 5%.

PET/MRI and PET/CT scan

Participants must fast for a minimum of 4 hours prior to the injection of FDG for the PET/MRI and PET/CT scans. However, they will be encouraged to drink water to ensure adequate hydration.

1. Upon arrival to department of nuclear medicine, the participant's height and weight must be measured using calibrated and medically-approved devices (not verbally collected from the participant). Serum glucose will be measured to determine that the blood glucose concentration is within the appropriate range
2. If the serum glucose concentration is found to be greater than 200 mg/dL, the study should be rescheduled. Before obtaining the PET scans, patients will have a large-bore (18-22 gauge) intravenous (IV) line placed. The FDG will be administered through the IV line.
3. For 15minutes before and 30min after injecting the FDG, the patient will rest in a warm room to avoid shivering and temperature effects that may increase muscular or fat uptake. The participant should move as little as possible and should not talk more than necessary during this time period.
4. The dose of FDG to be administered will be 10-15 millicuries (mCi), adjusted according to weight as per institutional norm within the range.
5. FDG will be synthesized and prepared in accordance with the United States Pharmacopeia (USP) compendial reference standards.
6. To standardize the scans, the scanning time for PET/MRI will be 2 hours +/- 10 minutes after FDG injection. The PET/CT will be performed 3 hours +/- 10 minutes after FDG injection.
7. Prior to positioning the participant on the PET scanners, the participant will be asked to urinate.

PET/MRI Imaging

1. PET/MRI scans will be performed on the Philips Ingenuity PET/MR scanner
2. A gadolinium based contrast agent will be used for the PET/MRI sequences. As mentioned above, GFR <75 ml/min/1.73m^2 based on the week-96 labs will be an exclusion criteria. The dose will be 0.1-0.2mmol/kg of body weight.
3. The patient will be fixed in a comfortable position on the scanner
4. Scout images will be obtained to localize the carotids and aorta
5. Standard sequences will be obtained for attenuation correction
6. Pre- and post-contrast multiplanar sequences will be obtained of the carotid arteries and aorta for the purposes of measuring vascular inflammation.
7. PET acquisition will occur as described in the PET/CT section after the MR sequences and will be timed so that the PET acquisition is 2 hours +/- 10 minutes after FDG injection.
8. Total time of PET/MRI scanning will be ~60 minutes

PET/CT Imaging

1. PET/CT scans will be performed on the Phillips Gemini TF Big Bore PET/CT

2. The participant should be placed in a comfortable position lying supine with the neck slightly extended.
3. Participants will undergo a dedicated head and neck PET acquisition in the standard arms down position. Images will be obtained from the orbits through the bottom of the heart in order to utilize the superior vena cava and right atrium to measure venous background SUV. The additional images will require one extra bed position for the PET scan and additional CT scan imaging.
4. A low-dose CT scan will be acquired for attenuation correction and anatomical localization of findings in the PET scan.
5. The CT scan will be performed during normal breathing. No respiratory gating will be applied.
6. PET/CT acquisition will occur 3 hours +/- 10 minutes after FDG injection.
7. Total time of PET/CT scanning will be ~10minutes

The standard uptake dose values (SUV) will be defined per standard convention as the decay-corrected tissue concentration of ^{18}F-FDG (in kBq/g) divided by the injected dose per body weight (in kBq/g).

The target-to-background ratio (TBR) will be measured offline for both PET/MRI and PET/CT. The left and right carotid artery will each be recorded separately. The target will be defined as either the aorta or the left/right carotid, and the background will be either: 1) superior vena cava (SVC), 2) right atrium or 3) venous blood sample radioactivity concentration.

To collect these values, there will be four regions of interest (ROI) that will be contoured after all. The aorta and each of the carotid arteries (left and right) will be contoured, as well as the SVC and the right atrium. To contour these ROIs, the PET scans will be fused with the MRI or CT. These five ROIs will also have an expansion to account for possible error. In each of the ROIs, the max, mean, and peak SUV will be recorded. From these recorded values, the TBR will be calculated.

The TBR will be calculated separately for the ascending aorta, left carotid, and right carotid by using the maximum and mean SUV of the vessel divided by the maximum and mean SUV of the 1) superior vena cava (SVC), 2) right atrium or 3) venous blood sample radioactivity concentration.

10. TOXICITY MANAGEMENT

Only toxicities related to study drugs (rosuvastatin) will be considered. Toxicities felt to be unrelated to study drugs will be left to the discretion of the primary care provider of the study participants.

AACTG grading system will be used for evaluation for Grading Adult Adverse Experiences, which can be found on http://rcc.tech-res-intl.com/tox_tables.htm
(Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004)

-Subjects who develop a new Grade 1 adverse event or toxicity may continue study drug without alteration of the dosage. Subjects experiencing Grade 1 adverse event who choose to withdraw from the study should be encouraged to complete the premature study evaluations as outlined in the Schedule of events table.

- For all toxicities of Grade 2 thought to be related to the study drug, the study medication

can be held at the discretion of the local investigator. If unable to resume it within 4 weeks, then the subject will be taken off study drugs but will continue to be followed and undergo the evaluations as per schedule of events.

- For all toxicities Grade 3 thought to be related to study drug, study medication will be held until the toxicity grade returns to ≤ Grade 2 or to the entry value. If unable to resume study drugs for longer than longer than 4 weeks, then the subject will be taken off study drugs but will continue to be followed and undergo the evaluations as per schedule of events.

- For any Grade 4 toxicity (confirmed X 2 values for laboratory abnormalities) regardless of the cause, subjects will be taken off study drugs but will continue to be followed and undergo the evaluations as per schedule of events. Exceptions are asymptomatic elevation of indirect bilirubin in subjects receiving indinavir or atazanavir therapy, asymptomatic elevation of CPK, or elevations in lipid levels.

- The specific management of toxicities believed to be related to the antiretrovirals will be left to the discretion of the primary care provider.

- Development of an acute coronary syndrome (e.g., acute MI, unstable angina) or stroke (e.g., ischemic, hemorrhagic, or embolic) should result in premature study drug discontinuation

- Specific notes related to key toxicities:

- If during the study, a subject develops signs on targeted physical exam (e.g. muscle swelling or tenderness) or possible symptoms of myositis or rhabdomyolysis (e.g. muscle aches, pain, weakness, or dark brown urine), CK should be performed as soon as possible but within 1 week
- Myopathy is defined as muscle aches, soreness, tenderness, or weakness with Grade □ 3 elevation in CK that is not related to exercise. If myopathy occurs, study medications should be permanently discontinued, and subjects will be followed off treatment, on study through week 96. CK will be added to the laboratory evaluations performed until it has declined to ≤ 1.0 X ULN.
- Rhabdomyolysis is defined as the presence of myopathy as above plus one or more of the following:
  - □ Hematuria on urine dipstick in the absence of microscopic hematuria (myoglobinuria).
  - □ Grade □ 2 hyperkalemia.
  - □ Grade □ 2 creatinine elevation.

  If rhabdomyolysis occurs, study medications should be permanently discontinued. Subjects will be followed closely off treatment, on study until week 96, and CK will be added to the laboratory evaluations performed until it has declined to ≤ 1.0 X ULN.

11. CRITERIA FOR PERMANENT STUDY DRUG DISCONTINUATION

- Drug-related toxicity (see section Toxicity Management).
- Clinical reasons believed life threatening by the physician, even if not addressed in the toxicity management of the protocol.
- Need to initiate open labeled statin medication
- Renal failure
- Liver failure
- Diagnosis of cardiovascular event (including myocardial infarction and

stroke)

CRITERIA FOR PERMANENT STUDY DISCONTINUATION

- Request by the subject to withdraw
- At the discretion of the FDA, IRB, or NIH
- Pregnancy or breast-feeding
- Subject judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol.
- Request of the primary care physician if s/he thinks the study is no longer in the best interest of the subject.

12. Statistical considerations

Sample Size/ Power Calculations Data from Hodis et al was used to estimate the expected difference in mean changes between the treated and placebo groups. A sample size of 56 per group would allow the detection of this difference (0.118) in mean changes as –significant‖ with >80% power. This sample size allows the detection of clinically relevant differences in changes for all outcome variables. We increased the sample size to 140 (70 per arm) to allow 20% loss to follow-up. All calculations presented are for 2-sided testing. Calculations were done using □=0.05 but the table shows that we will be able to adjust the level of significance for multiple uses of the data. The addition of 5 or fewer covariates should reduce power by <10 %.

Variable	Specific Aim	Difference in mean change	Standard deviation	Power
FMD%	1	-3.300	0.800	>0.99
cca IMT	1	0.118	0.208	>0.84
Calcium score	1	12%	12%	>0.99
Sharp	3	6.000	7.300	>0.99
F2 isoprostanes	3	0.018	0.027	>0.93
Spine BMD	2	-0.027	0.036	>0.97
CTX	2	Difference in median percent change > 0.25		>0.99

Statistical Analysis Plan. Statistical analysis of a cleaned and locked master database will be conducted ath the end of the study.. An interim analysis will also be conducted after all participants complete weeks 12 and 24 and will look at inflammation and oxidative markers. All statistical analyses will be done using SAS statistical software (SAS Institute Inc., Cary, NC, USA, Proprietary Software Release 9.2, 2007).

The principal analyses will employ the intent-to-treat approach, thus including all participants as belonging to their original group; treatment group membership will not be altered based on the participant's adherence to that treatment. All statistical tests will be two-sided. Adjusted p-values will be reported for the main outcome variables, nominal ones for secondary outcomes. In general, continuous variables will be summarized with standard descriptive statistics including means, standard deviations, medians, and ranges. Categorical variables will be summarized with frequencies and percentages; 99%

confidence intervals will be provided for descriptive statistics, as warranted. Markedly non- normally distributed data will be transformed prior to inferential comparisons. For variables
that cannot be successfully transformed, nonparametric methods will be used, recognizing
that this approach will be more limited than those outlined below for normally distributed outcome data. Model assumptions will be checked with standard model diagnostics.

The primary outcome variables for each aim are as follows. SA1: FMD, cIMT, and calcium scores; SA2: Spine BMD, and CTX; SA3: hsCRP, and F2-isoprostanes. In the main analysis of the hypotheses set forth in each specific aim, the outcome measure will be the absolute change of the respective outcome variable between baseline and 96 weeks (48 weeks from FMD). Analysis of covariance will be utilized to express that change as a function of treatment, with the baseline value of the outcome variable as the covariate. In secondary analyses, we will adjust for other relevant covariates. Interactions between treatment and other key factors will be assessed. Additional analyses will focus on percent change between baseline and 96 weeks as the outcome variable. Pearson's correlation coefficient will be used to examine the (linear) association of markers of inflammation (and of oxidation) with FMD, IMT, calcium scores, BMD and bone turnover. In order to support the primary findings, since data are longitudinal in nature, two other approaches will be taken, both focused on rates of change. In one of them, just exploratory, individual rates of change will be estimated by the slope of each individual's least-squares regression; mean rates of change will then be compared between the 2 treatment groups using analysis of covariance with baseline values as the covariate. The other approach will use SAS PROC MIXED to develop a random effects model of mean rates of change across individuals. This approach will take into account time-dependent covariates, such as changes in ART. While missing data is not a particular concern in this study, it should be mentioned that the second approach outlined requires that few outcome values will be missing, and that the missingness be completely at random (MCAR).

13. HUMAN SUBJECTS

13.1. Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the CWRU IRB. A signed informed consent form will be obtained from each study subject. The informed consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, and this fact will be documented in the subject's record.

13.2. Subjects and Data Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRB, FDA, OHRP, or NIH and AstraZeneca.

13.3. Recruitment plans

Subjects will be primarily recruited from the practice of the investigator (the Special Immunology Unit at University Hospitals Case Medical Center). All subjects seen at the Special Immunology Unit who meet the inclusion/exclusion criteria will be approached for study participation. In addition, subjects may be referred to the study by their primary care

provider or their HIV provider from other HIV practices.

13.4. Study Discontinuation

The study may be discontinued at any time by the IRB, FDA or NIH as part of their duties to ensure that research subjects are protected.

13.5. Post-study Follow-up and Transition of Care

During the study period, the study subject will be encouraged to continue their routine regular follow up with their primary HIV care provider, whether in the Special Immunology Unit or in an outside HIV practice that referred them to the study. The study is not meant to replace the routine HIV care visits. After the study subject completes the study, he/she will continue to be followed by his/her primary HIV provider. There will be no additional follow-up for this study after the 96-week of the pre-defined study period. In addition, after the study subject completes the study, he/she will not be provided any further study drugs. The study drug rosuvastatin is FDA-approved and available in the USA. If the subject primary care provider decides that it is in the best interest of the patient to be on rosuvastatin, he/she would prescribe it to the study subjects. The decision related to any follow-up or treatment after the study period will be left to the subject and his/her primary care provider.

14. BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All infectious specimens will be packaged and sent in accordance with requirements mandated by the International Air Transport Association Dangerous Goods Regulations-Packing Instruction 602.

15. DATA SAFETY AND MONITORING PLAN

A Data Safety and Monitoring plan (DSMB) will be established by the Research Core to oversee the conduct of this study to ensure participant safety and quality of the data. The DSMB will be appointed to oversee data collection and the intervention activities at all sites. The membership of the DSMB will be completely independent from the study team. The DSMB will function to: review and approve plans for data and safety monitoring for this trial; to review data on a timely basis and to ensure proper conduct and progress of study; to review credentials of investigators, project staff, and consultants; to make recommendations to project investigators and staff regarding issues of concern; to address adverse events. The DSMB will meet twice a year in Cleveland, Ohio or by phone to review study protocols, progress, and data. Meetings will be held in an open format, except if privileged data are discussed. Dr McComsey is responsible for developing site reports and materials for dissemination for the DSMB.

The Principal Investigator will report all ≥ grade 3 adverse effects deemed to be possibly, probably or definitely related to study participation to the CWRU IRB, FDA, and NIH and AstraZeneca within one week in writing. The Principal Investigator will notify FDA,NIH and AstraZeneca of any action recommended by the CWRU IRB that may affect the conduct of the trial. All grade ≥ 1 adverse effects deemed to be possibly, probably or definitely related to study participation will be collected. The Principal Investigator, investigators, and study nurse will prepare every year an update to renew the CWRU IRB approval of the clinical studies. In this renewal, investigators will inform the IRB about

adverse effects grade > 1 noted during the performance of the study. Accrual, retention, and data quality and timeliness will be monitored by the Principal Investigator.

Adverse Events

Definitions

The definitions of Adverse Events (AEs) and Serious Adverse Events (SAEs) are given below. It is of the utmost importance that all staff involved in the study be familiar with the content of this section. The principal investigator is responsible for ensuring this.

Adverse Event
An Adverse Event (AE) is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, ECG). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.
Any detrimental change in a patient's condition subsequent to them entering the study and during the follow-up period should be considered an AE. When there is a deterioration in the condition for which the study treatment is being used, there may be uncertainty as to whether this is lack of efficacy or an AE. In such cases, unless the reporting physician considers that study treatment contributed to the deterioration or local regulations state to the contrary, the deterioration should be considered a lack of efficacy. Signs and symptoms of disease progression are therefore not considered AEs.

Serious Adverse Event

A Serious Adverse Event (SAE) is an AE occurring during any study phase (eg, run-in, treatment, washout, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfills one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above.

Any event or hospitalization that is unequivocally due to progression of disease, as determined by the investigator, must not be reported as an SAE; however, the event should be communicated to AstraZeneca

The causality of SAEs (their relationship to all study treatment) will be assessed by the investigator(s) and communicated to AstraZeneca.

Reporting of Adverse Events
When recording/reporting AEs, the use of diagnoses is preferred (when possible) to recording/reporting a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded/reported separately.

Investigators and other site personnel must inform the FDA, via a MedWatch/AdEERS form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations of 21CFR312.32, and will concurrently forward all such reports to AZ.

A copy of the MedWatch/AdEERS report must be faxed to AstraZeneca at the time the event is reported to the FDA. It is the responsibility of the investigator to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca at the same time.

* A *cover page* should accompany the *MedWatch/AdEERS* form indicating the following:

- *Crestor Investigator Sponsored Study (ISS)*
- The *investigator's name* and *address*
- The *trial name/title* and *AstraZeneca reference number*

* Investigative site must also indicate, either in the SAE report or the cover page, the *causality* of events *in relation to all study medications* and if the SAE is *related to disease progression*, as determined by the principal investigator.

* *Send SAE report and accompanying cover page by way of fax to AstraZeneca's designated fax line: (866) 984-7229, Attention CRESTOR ISS Safety Representative*
If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to AstraZeneca and the FDA.

Serious adverse events that do not require expedited reporting to the FDA need to be reported to AstraZeneca preferably using the MedDRA coding language for serious adverse events. This information should be reported on a monthly basis and under no circumstance less frequently than quarterly.
In the case of blinded trials, AstraZeneca will request that the Sponsor either provide a copy of the randomization code / code-break information or unblind those SAEs which require expedited reporting.

All SAEs have to be reported to AstraZeneca, whether or not considered causally related to the investigational product. All SAEs will be documented. The investigator is responsible for informing the IRB and/or the Regulatory Authority of the SAE as per local requirements.

Non-serious adverse events and SAEs will be collected from the time consent is given, throughout the treatment period and up to and including the *30 day follow-up* period. After withdrawal from treatment, subjects must be followed-up for all existing and new AEs for *30 calendar days after the last dose of trial drug and/or until event resolution.* All new AEs occurring during that period must be recorded (if SAEs they must be reported to the FDA and AstraZeneca). All study-related toxicities / SAEs must be followed until resolution, unless in the Investigator's opinion, the condition is unlikely to resolve due to the patient's underlying disease.

16. Illiterate Subjects

Subjects with all levels of literacy will be eligible for this study. The consent document will be read to those volunteers with less than an 8th grade equivalent level of literacy.
Subsequently, the informed consent will be signed by the volunteer making their –mark‖ in the signature section in order to document their understanding. A witness will be present to confirm the consent process has taken place. Both the witness and person conducting

the consent process will sign and date the consent. The investigator obtaining consent will ask each subject to reiterate what will be required from them, risks and benefits, and their rights as a participant in order to ensure their full understanding of the study.

17. Non-English Speaking Subjects

Subjects who do not understand or speak English will also be eligible for this study. The consent form will be read to those non-english speaking study candidates in their primary language by a translator from the University Hospitals of Cleveland translation/International Visitors Center. A witness (who speaks English and the study subject's language) will be present to confirm the consent process has taken place. Both the witness and person conducting the consent process will sign and date the consent. The investigator obtaining consent will ask the study candidate via the translator to reiterate what will be required from him/her, risks and benefits, and his/her rights as a participant in order to ensure their full understanding of the study.

18. BENEFITS

There may be no direct benefit to patients from participating in this study. Their participation in the study will allow collection of valuable information about the study medication in persons infected with the HIV virus. This information may be useful to them and other people with HIV disease.

19. ALTERNATIVES

Alternatives to participation in this study are not to participate and to receive the standard of care from the patient's primary care doctor.

20. COST TO PARTICIPANTS

There is no cost to participants for the study related clinic visits, examinations or laboratory test required by this study. Medical costs of other treatment or examinations outside of the study will be the responsibility of the patient or their insurance company. Patients will be provided study drugs free of charge for the entire duration of the study. The study drug will not be provided after they finish the study (whether they complete the study or prematurely stop study participation).

21. PAYMENT TO SUBJECTS

For completed study visits screening, week 6, 12, 24, 36, and 72 patients will receive $25.00 that day, and for completed study visits at entry, week 48 and 96 they will receive 50$ that day (total of $300 for completing the entire study). This payment will help cover the expense of childcare, transportation and time off work that patients may incur as a result of being in this study. To help with the cost of gas, for participants traveling $\geq$ 20-40 miles one way for their appointments, they will also be given a $15.00 gas card to cover the cost of the transportation. Participants traveling $\geq$ 40 miles one way for their appointments will also be given a $30.00 gas card to cover the cost of transportation.

For those who participate in the onetime optional ECHO, patients will receive an additional $15.00 once the ECHO is completed.

For those who participate in the onetime optional PET/CT; PET/MR, patients will receive an additional $75.00 once the visit is completed.

22. RISKS AND DISCOMFORTS:

The risks for taking part in this study are:

Blood Draw

Risks associated with drawing blood include: pain, bleeding, and bruising at the site of the blood draw. Other rare risks include: lightheadedness and/or fainting or infection at the site.

IMT test

Because nothing physical passes through patients' skin and the sound waves used do not expose patients to any x-rays or other type of potentially dangerous energy, there are no risks relating to having the IMT test performed.

Flow mediated dilation

It is a painless imaging test that has no short or long-term risks. The test may be mildly to moderately uncomfortable because of the blood pressure cuff that is applied to the patients' arm tightly.

DEXA Scan

This test involves a small amount of radiation. To give patients an idea about how much radiation they will get, we will compare it to the amounts that people encounter in daily life. There is radiation that naturally occurs from space and from rocks in the soil. This natural radiation is greater at higher altitudes. This research gives patients about the same amount of radiation as they would get from living in a high altitude city such as Denver for 6 days, or taking 2 airplane flights from New York to Los Angeles. The radiation dose we have discussed is what patients will receive from this study only and does not include any exposure they may have received or will receive from other tests.

CT scan for coronary calcium scoring and epicardial fat:

If you take part in this research, you will have one or more medical imaging studies which use radiation. The tests or treatments you will have include a cardiac calcium score scan. To give you an idea about how much radiation you will get, we will make a comparison with an every-day situation. Everyone receives a small amount of unavoidable radiation each year. Some of this radiation comes from space and some from naturally-occurring radioactive forms of water and minerals. This research gives your body the equivalent of about 4 extra years' worth of this natural radiation. The radiation dose we have discussed is what you will receive from this study only and does not include any exposure you may have received or will receive from other tests.

Rosuvastatin

Possible side effects include:

- Upset stomach, vomiting, heartburn, diarrhea or constipation, or abdominal pain
- Muscle pain or joint pain
- Allergic reaction is very rare, but may include: rash, swelling of the face, shortness of breath and anaphylaxis (serious allergic reaction that leads to very low blood pressure, difficulty breathing and may result in death)
- In less than 1% of patients, liver toxicity

If patients develop any of the above symptoms or any other symptom that they feel may be related to the study medication, they are informed to call the study investigator at 216-844-7890 and ask for Dr McComsey.

Pregnancy

Rosuvastatin is not safe for unborn babies. If patients are having sex that could lead to pregnancy, they must agree not to become pregnant or make a woman pregnant. Because of the risk involved, patients and their partner must use two methods of birth control that they discuss with the study staff. They must continue to use both methods until 6 weeks after stopping study drug. They may choose two of the birth control methods listed below:

- Birth control drugs that prevent pregnancy given by pills, shots, intra-vaginal ring or placed on or under the skin
- Male or female condoms with or without a cream or gel that kills sperm
- Diaphragm or cervical cap with a cream or gel that kills sperm
- Intrauterine device (IUD)

If women can become pregnant, they must have a pregnancy test before they enter this study. The pregnancy test must be negative. In addition, is study subjects think they may be pregnant at any time during the study, they are to tell the study staff right away. In the event that a patient becomes pregnant while on study, they will be taken off study, and no further evaluations or tests will be performed as part of the study.

Placebo

If patients are assigned to placebo, they will go without active study drug, but they will continue to receive their prescribed anti-HIV medication.

Fasting

Some individuals find fasting to be bothersome. It may make some individuals feel anxious, irritable, or hungry. Patients who are required to take their morning medications with food should wait until after the visit has been completed to take their medications.

Anthropometric Measurements

No calipers will be used. A tape measure will be used to get your waist and hip circumference; this tape measure may be cold. Some individuals find minimal discomfort in exposing their waist and hips.

PET/CT and PET/MRI

Some people cannot have an MRI because they have some type of metal in their body. For instance, if they have a heart pacemaker, artificial heart valves, metal implants such as metal ear implants, bullet pieces, chemotherapy or insulin pumps or any other metal such as metal clips or rings, they cannot have an MRI. During this test, the participant will lie in a small closed area inside a large magnetic tube. Some people are scared or anxious in small places (claustrophobic). The MRI scanner makes loud banging noises while taking a measurement, so either ear plugs or specially designed headphones will be used to reduce the noise.

The use of gadolinium-based contrast agents in patients who already have severely reduced kidney function is uncommonly (less than 10%) associated with a possibly fatal disease involving the skin, muscle and internal organs. This disorder is called nephrogenic systemic fibrosis (NSF). As stated in the earlier sections of the consent form, the participant will have blood drawn to test their kidney function and will be excluded if they have history of severe kidney problems or have had a kidney and/or liver transplant. The dose and frequency of the contrast agent is standard. We do not expect any additional risk to be posed to the participant beyond those already described above.

The cumulative radiation exposure from these tests is considered small and is not likely to adversely affect their disease. However, the effects of radiation add up over a lifetime.

OTHER RISKS

Patients are informed to tell their study doctor or study nurse about all other drugs they are currently taking including non-prescription medications, alcohol, recreational, and herbal products. These drugs, if taken with the study medication, can result in dangerous interactions. If patients have questions about the drug that will be used in this study and the potential for interaction with other drugs that they take, they are instructed to ask their study doctor to provide additional information.

In addition to the risks and discomforts listed here, there may be others that are currently not known.

REFERENCES

1. Hogg R and ART Cohort Collaboration. Life expectancy of persons at the time of initiating cART in high-income countries. 14th Conference on Retroviruses and Opportunistic Infections. February 25-28, 2007. Los Angeles. Abstract 972
2. Friis-Moller N, Reiss P, Sabin CA, Weber R, Monforte A, El Sadr W, Thiebaut R, De Wit S, Kirk O, Fontas E, Law MG, Phillips A, Lundgren JD. Class of antiretroviral drugs and the risk of myocardial infarction. N Engl J Med 2007;356:1723-35
3. McComsey GA, O'Riordan M, Hazen SL, El-Bejjani D, Bhatt S, Brennan ML, Storer N, Adell J, Nakamoto DA, Dogra V. Increased carotid intima media thickness and cardiac biomarkers in HIV infected children: Good reasons for concern. AIDS 2007;21(8):921-927
4. Johnsen S, Dolan SE, Fitch KV, Kanter JR, Hemphill LC, Connelly JM, Lees RS, Lee H, Grinspoon S. Carotid Intimal Medial Thickness in HIV Infected Subjects: Effects of Protease Inhibitor Use, Cardiac Risk Factors and the Metabolic Syndrome. The Journal of Clinical Endocrinology & Metabolism 2006;91(12):4916-4924
5. Hsue PY, Lo JC, Franklin A, Bolger AF, Martin JN, Deeks SG, Waters DD. Progression of Atherosclerosis as Assessed by Carotid Intima-Media Thickness in Patients With HIV Infection. Circulation 2004; 109:1603-1608
6. Mercie P, Thiebaut R, Lavignolle V, Pellegrin JL, Yvorra-Vives MC, Morlat P, Ragnaud JM, Dupon M, Malvy D, Bellet H, Lawson-Ayayi S, Roudaut R, Dabis F. Evaluation of cardiovascular risk factors in HIV-1 infected patients using carotid intima-media thickness measurement. Ann Med 2002;34:55-63
7. Bongiovanni M, Casana M, Cicconi P, Pisacreta M, Codemo R, Pelucchi M, d'Arminio Monforte A, Bini T. Predictive factors of vascular intima media thickness in HIV-positive subjects. J Antimicrob Chemother. 2008;61(1):195-9.
8. Currier JS, Kendall MA, Zackin R, Henry WK, Alston-Smith B, Torriani FJ, Schouten J, Mickelberg K, Li Y, Hodis HN. Carotid artery intima-media thickness and HIV infection: traditional risk factors overshadow impact of protease inhibitor exposure. AIDS 2005;19:927-33
9. Strategies for Management of Antiretroviral Therapy (SMART) Study Group, El-Sadr WM, Lundgren JD, et al. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med. 2006;355(22):2283-2296
10. Torriani FJ, Parker RA, Murphy FL, Stein J. Antiretroviral therapy improves endothelial function in treatment-naive HIV-infected subjects: a prospective, randomised multicenter trial (A5152S). The 10th European AIDS Conference. November 17-20, 2005. Dublin. Abstract PS5/3
11. Wolfrum S, Jensen KS, Liao JK. Endothelium-dependent effects of statins. Arterioscler Thromb Vasc Biol 2003;23(5):729-73.
12. Davignon J. Beneficial cardiovascular pleiotropic effects of statins. Circulation 2004;109(23 Suppl 1):III39-43
13. Tsunekawa T, Gayashi T, Kano H, Sumi D, Matsui-Hirai H, Thakur NK, Egashira K, Iguchia A. Cerivastatin, a hydroxymethylglutaryl coenzyme a reductase inhibitor, improves endothelial function in elderly diabetic patients within 3 days. Circulation 2001;104(4):376-9.
14. Pirro M, Schillaci G, Romagno PF, Mannarino MR, Bagaglia F, Razzi R, Pasqualini L, Vaudo G, Mannarino E. Influence of Short-term Rosuvastatin Therapy on Endothelial Progenitor Cells and Endothelial Function. J Cardiovasc Pharmacol Ther 2009;14(1):14-21

15. Dogra GK, Watts GF, Herrmann S, Thomas MA, Irish AB. Statin therapy improves brachial artery endothelial function in nephrotic syndrome. Kidney Int 2002; 62(2):550-557
16. Amudha K, Choy AM, Mustafa MR, Lang CC. Short-term effect of atorvastatin on endothelial function in healthy offspring of parents with type 2 diabetes mellitus. Cardiovasc Ther 2008;26(4):253-261
17. Holschermann H, Schuster D, Parviz B, Haberbosch W, Tillmanns H, Muth H. Statins prevent NF-kappaB transactivation independently of the IKK-pathway in human endothelial cells. Atherosclerosis 2006;185(2):240-5
18. E. Ben-Tal Cohen, P.J. Hohensinner, C. Kaun, G. Maurer, K. Huber and J. Wojt. Statins decrease TNF-α-induced osteoprotegerin production by endothelial cells and smooth muscle cells in vitr.o Biochemical pharmacology 20007; 73(1):77-8
19. Valent P, Müller MR, Husslein P; Kashanipour A, Minar E, Baghestanian M Simvastatin Reduces Expression of Cytokines Interleukin-6, Interleukin-8, and Monocyte Chemoattractant Protein-1 in Circulating Monocytes From Hypercholesterolemic Patients. Arteriosclerosis, Thrombosis, and Vascular Biology. 2002;22
20. Bonnet J, McPherson R, Tedgui A, Simoneau D, Nozza A, Martineau P, Davignon J; CAP Investigators.Comparative effects of 10-mg versus 80-mg atorvastatin on high-sensitivity C-reactive protein in patients with stable coronary artery disease: results of the CAP study. Clin Ther. 2008;30(12):2298-313
21. Oka H, Ikeda S, Koga S, Miyahara Y, Kohno S. Atorvastatin induces associated reductions in platelet P-selectin, oxidized low-density lipoprotein, and interleukin-6 in patients with coronary artery diseases. Heart Vessels 2008;23(4):249-56
22. Usharani P, Mateen AA, Naidu MU, Raju YS, Chandra N. Effect of NCB-02, atorvastatin and placebo on endothelial function, oxidative stress and inflammatory markers in patients with type 2 diabetes mellitus: a randomized, parallel-group, placebo-controlled, 8-week study. Drugs RD. 2008;9(4):243-250
23. Ridker PM, Danielson E, Fonseca FA,Genest J Jr, Gotto AM, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFayden JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ, Jupiter Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008;359(21):2195-207
24. Espeland MA, O'Leary DH, Terry JG, Morgan T, Evans G, Mudra H. Carotid intimal-media thickness as a surrogate for cardiovascular disease events in trials of HMG-CoA reductase inhibitors. Curr Control Trials Cardiovasc Med. 2005; 6(1): 3
25. Sugiyama M again Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu C, Liu C, Alaupovic P, Kwong-Fu H, Azen SP. Reduction in Carotid Arterial Wall Thickness Using Lovastatin and Dietary Therapy: A Randomized, Controlled Clinical Trial. Ann Intern Med 1996;124(6):548-556
26. Smilde TJ, van Wissen S, Wollersheim H, Trip MD, Kastelein JJP, Stalenhoef AFH. Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolaemia(ASAP): a prospective, randomised, doubleblind trial. Lancet 2001;357:577-81
27. Crouse JR 3rd, Raichlen JS, Riley WA, Evans GW, Palmer MK, O'Leary DH, Grobbee DE, Bots ML; METEOR Study Group. Effect of rosuvastatin on progression of carotid intima-media thickness in low-risk individuals with subclinical atherosclerosis: the METEOR Trial. JAMA 2007;297(12):1344-53.
28. Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, Boyce B, Zhao M, Gutierrez G: Stimulation of bone formation in vitro and in rodents by statins. Science 1999, 286:1946-1949
29. Maeda T, Matsunuma A, Kawane T, Horiuchi N: Simvastatin promotes osteoblast differentiation and mineralization in MC3T3-E1 cells. Biochem Biophys Res

Commun 2001, 280**:**874-877
30. Ohnaka K, Shimoda S, Nawata H, Shimokawa H, Kaibuchi K, Iwamoto Y, Takayanagi R: Pitavastatin enhanced bmp-2 and osteocalcin expression by inhibition of rho-associated kinase in human osteoblasts. Biochem Biophys Res Commun 2001, 287**:**337-342
31. Dimmeler S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, Rutten H, Fichtlscherer S, Martin H, Zeiher AM: HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. J Clin Invest 2001, 108:391-397
32. Vasa M, Fichtlscherer S, Adler K, Aicher A, Martin H, Zeiher AM, Dimmeler S: Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. Circulation 2001, 103:2885-2890
33. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N: VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat Med 1999, 5:623-628
34. Yamashita M, Otsuka F, Mukai T, Otani H, Inagaki K, Miyoshi T, Goto J, Yamamura M, Makino H. Simvastatin antagonizes tumor necrosis factor-alpha inhibition of bone morphogenetic proteins-2-induced osteoblast differentiation by regulating Smad signaling and Ras/Rho-mitogen-activated protein kinase pathway. J Endocrinol. 2008;196(3):601-13
35. Ahn KS, Sethi G, Chaturvedi MM, Aggarwal BB. Simvastatin, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, suppresses osteoclastogenesis induced by receptor activator of nuclear factor-kappaB ligand through modulation of NF-kappaB pathway. Int J Cancer 2008; 123(8):1733-40
36. Viereck V, Grundker C, Blaschke S, Frosch KH, Schoppet M, Emons G, Hofbauer LC. Atorvastatin stimulates the production of osteoprotegerin by human osteoblasts. J Cell Biochem 2005;96(6):1244-53.
37. Edwards CJ, Hart DJ, Spector TD: Oral statins and increased bone-mineral density in postmenopausal women. Lancet 2000, 355**:**2218-2219
38. Chan KA, Andrade SE, Boles M, Buist DS, Chase GA, Donahue JG, Goodman MJ, Gurwitz JH, LaCroix AZ, Platt R: Inhibitors of hydroxymethylglutaryl-coenzyme A reductase and risk of fracture among older women. Lancet 2000, 355:2185-2188
39. Wang PS, Solomon DH, Mogun H, Avorn J: HMG-CoA reductase inhibitors and the risk of hip fractures in elderly patients. JAMA 2000, 283**:**3211-3216
40. Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H**:** HMG-CoA reductase inhibitors and the risk of fractures. JAMA 2000, 283:3205-3210
41. Reid IR, Hague W, Emberson J, Baker J, Tonkin A, Hunt D, MacMahon S, Sharpe N: Effect of pravastatin on frequency of fracture in the LIPID study: secondary analysis of a randomized controlled trial. Long-term Intervention with Pravastatin in Ischaemic Disease. Lancet 2001, 357:509-512
42. van Staa TP, Wegman S, de Vries F, Leufkens B, Cooper C: Use of statins and risk of fractures. JAMA 2001, 285:1850-1855
43. Majima T, Shimatsu A, Komatsu Y, Satoh N, Fukao A, Ninomiya K, Matsumura T, Nakao K. Short-term effects of pitavastatin on biochemical markers of bone turnover in patients with hypercholesterolemia. Intern Med. 2007;46(24):1967-73
44. Majima T, Komatsu Y, Fukao A, Ninomiya K, Matsumura T, Nakao K. Short-term effects of atorvastatin on bone turnover in male patients with hypercholesterolemia. Endoc J 2007; 54(1):145-151
45. Berthold HK, Unverdorben S, Zittermann A, Degenhardt R, Baumeister B, Unverdorben M, Krone W, Vetter H, Gouni-Berthold I. Age-dependent effects of atorvastatin on biochemical bone turnover markers: a randomized controlled trial in postmenopausal women. Osteoporos Int. 2004 Jun;15(6):459-67
46. Johns KW, Bennett MT, Bondy GP. Are HIV positive patients resistant to statin therapy? Lipids Health Dis. 2007; 6: 27

47. Calza, L; Colangeli, V; Manfredi, R; Legnani, G; Tampellini, L; Pocaterra, D; Chiodo, F. Rosuvastatin for the treatment of hyperlipidaemia in HIV-infected patients receiving protease inhibitors: a pilot study. AIDS 2005;19:1103–5
48. Calza L, Manfredi R, Colangeli V, Pocaterra D. Pavoni M, Chiodo F. Rosuvastatin, pravastatin, and atorvastatin for the treatment of hypercholesterolaemia in HIV-infected patients receiving protease inhibitors. Curr HIV Res 2008; 6(6):572-8
49. Hurlimann D, Chenevard R, Ruschitzka F, et al. Effects of statins on endothelial function and lipid profile in HIV infected persons receiving protease inhibitor–containing antiretroviral combination therapy: a randomized double-blind cross-over trial. Heart 2006; 92:110–2
50. Stein JH, Merwood MA, Bellehumeur JL, et al. The effects of pravastatin on atherogenic lipoproteins and endothelial function in patients with human immunodeficiency virus. Am Heart J 2004; 147:1–7
51. Roberts LJ, Morrow JD. Measurements of F_2-isoprostanes as an index of oxidative stress in vivo. Free Radic Biol Med 2000;28: 505-513
52. Morrow JD. The isoprostanes: their quantification as an index of oxidant stress Hodis status in vivo. Drug Metab Rev 2000;32:377-385
53. Morrow JD, Roberts LJ. Mass spectrometric quantification of F2-isoprostanes in biological fluids and tissues as measure of oxidant stress. Meth. Enzymol. 1999;300: 3-12
54. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar S, Rembold CM, Post WS. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: A consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Journal of the American Society of Echocardiography. 2008;91:93-111
55. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab 2007 Jul;92(7):2506-12.
56. Hsue PY, Hunt PW, Ho JE, et al. Impact of HIV infection on diastolic function and left ventricular mass. Circ Heart Fail 2010 Jan;3(1):132-9.
57. Mondy KE, Gottdiener J, Overton ET, et al. High Prevalence of Echocardiographic Abnormalities among HIV-infected Persons in the Era of Highly Active Antiretroviral Therapy. Clin Infect Dis 2011 Feb 1;52(3):378-86.
58. Sitia S, Tomasoni L, Cicala S, et al. Detection of preclinical impairment of myocardial function in rheumatoid arthritis patients with short disease duration by speckle tracking echocardiography. Int J Cardiol 2012; 160:8-14.
59. Buss SJ, Wolf D, Korosoglou G, et al. Myocardial Left Ventricular Dysfunction in Patients with Systemic Lupus Erythematosus: New Insights from Tissue Doppler and Strain Imaging. J Rheumatol 2010; 37:79-86.
60. Fine NM, Shah AA, Han IY, et al. Left and right ventricular strain and strain rate measurement in normal adults using velocity vector imaging: an assessment of reference values and intersystem agreement. Int J Cardiovasc Imaging 2012 Sep 14.
61. Libby P, Ridker PM, Hansson GK, Leducq Transatlantic Network on A. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol* 2009,54:2129-2138.
62. Hsue PY, Deeks SG, Hunt PW. Immunologic basis of cardiovascular disease in HIV-infected adults. *J Infect Dis* 2012,205 Suppl 3:S375-382.

63. Subramanian S, Tawakol A, Burdo TH, Abbara S, Wei J, Vijayakumar J*, et al.* Arterial inflammation in patients with HIV. *JAMA* 2012,308:379-386.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	4
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	4
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	4;5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	4
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	7
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	4;7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	4;8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	4;8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	8

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	7
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	7;8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	8
	13b	For each group, losses and exclusions after randomisation, together with reasons	8
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	8
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	8
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	8;9
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	8;9
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12,13
Other information			
Registration	23	Registration number and name of trial registry	4
Protocol	24	Where the full trial protocol can be accessed, if available	8
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	1

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram