Rationale and design of the Mechanistic Substudy of the Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE): Effects of pitavastatin on coronary artery disease and inflammatory biomarkers

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on behalf of the REPRIEVE Investigators

Abstract Background People with HIV (PWH) have increased cardiovascular events, inflammation, and high-risk coronary atherosclerosis. Statin therapy has been shown to lower the risk of cardiovascular disease (CVD) in the general population, but whether this results from reductions in coronary atherosclerosis and is mediated by decreased inflammation remains unknown.

Methods REPRIEVE is a randomized, placebo-controlled trial of pitavastatin calcium (4 mg/day) vs. placebo enrolling at least 7500 PWH between 40–75 years, on antiretroviral therapy (ART), with low to moderate traditional CVD risk. The Mechanistic Substudy of REPRIEVE (A5333s) is co-enrolling 800 participants from 31 US sites. These participants undergo serial contrast enhanced coronary computed tomography angiography (CCTA) and measurements of biomarkers of inflammation and immune activation at baseline and after 2 years of follow-up. The primary objectives are to determine the effects of pitavastatin on noncalcified coronary atherosclerotic plaque (NCP) volume, low attenuation plaque, and positive remodeling and on changes in immune activation and inflammation and to assess relationships between the two. Changes in CAD will be assessed in a standardized fashion by a core lab with expert readers blinded to time points and participant information; immune activation and inflammation assessment is also performed centrally.

Results To date the Mechanistic Substudy has completed planned enrollment, with 805 participants.

Conclusion This study represents the first large, randomized, CCTA-based assessment of the effects of a primary prevention strategy for CVD on high-risk CAD, immune activation and inflammation among PWH. The study will assess pitavastatin's effects on coronary plaque, and the interrelationship of these changes with biomarkers of immune activation and inflammation in PWH to determine mechanisms of CVD prevention and improved outcomes in this population. (Am Heart J 2019;212:1-12.)

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People with HIV (PWH) have an excess 1.5- to 2-fold risk of myocardial infarction (MI) as compared to HIV-uninfected individuals with a similar traditional cardiovascular (CV) risk profile.[1,2] This increased risk is thought to be due, in part, to immune dysfunction (e.g. reduced CD4+ T-cell counts) and to heightened immune activation and inflammation related to the virus, both of which may lead to acceleration of high-risk coronary artery disease (CAD), even in young PWH with relatively low traditional risk scores.[3,4] Moreover, PWH have demonstrated increased noncalcified and high risk plaque[5-7] suggesting increased vulnerable plaque as a mechanism of cardiovascular disease (CVD) HIV. Statins reduce major adverse cardiovascular events (MACE) in the HIV-uninfected population[8] and have been shown to effectively reduce LDL cholesterol (LDL-C), key inflammatory markers, immune activation indices among HIV-infected patients.[9,10] For example, 48 weeks of rosuvastatin treatment reduced significantly several markers of inflammation and lymphocyte and monocyte activation in ART-treated subjects.[11] However, results have varied depending on endpoints and study design and a recent meta-analysis of studies in HIV positive patients could not identify a clear association between any of the immune markers and surrogate CVD outcomes.[12] This may be due to insensitivity of the primary surrogate markers used (CAC and arterial stiffness) or may be explained by heterogeneity across studies and lack of follow-up data. Overall though, it emphasizes the need for a well powered and conducted study to establish an association and moreover provide preliminary data on whether this association drives outcomes.

The REPRIEVE is a landmark trial to determine whether statin therapy (in this case pitavastatin) will prevent MACE in HIV-infected patients with low to moderate traditional CVD risk. An important aspect of REPRIEVE is the embedded Mechanistic Substudy of REPRIEVE (A5333s), hereafter referred to as the Mechanistic Substudy, that will simultaneously evaluate the mechanisms by which statins achieve an effect in PWH. This substudy will test whether statin therapy can reduce high-risk coronary plaque, modify inflammation and immune activation and the association of these effects with each other and with adverse cardiovascular outcomes.

Recent data in HIV demonstrate increased immune activation (monocyte activation, T cell activation), generalized inflammation, and arterial inflammation, as well as increased coagulation markers. Moreover, among PWH key indices of systemic immune activation and inflammation (including monocyte activation markers such as soluble CD14 (sCD14), soluble CD163 (sCD163) and T-cell activation markers) have been linked to subclinical carotid atherosclerosis, high-risk coronary atherosclerotic plaque, cardiovascular events, and mortality.[6,9,13-21] Importantly, among PWH, ART lowers systemic immune activation, but may not be adequate to effectively reduce ongoing systemic and arterial inflammation and atherogenesis,[22-24] highlighting the potential importance of adjunctive anti-inflammatory strategies for CVD prevention.[12,25] Statin effects on arterial inflammation, oxidized LDL (oxLDL), sCD14, CRP, TNF-alpha, and immune activation in association with reductions in high-risk plaque (HRP), may provide a potential mechanism for statin effects on MACE.[9,11,26-29]

Coronary computed tomography angiography (CCTA) allows for non-invasive and accurate characterization of CAD, including atherosclerotic plaque and luminal stenosis.[30] Across populations, both non-obstructive and obstructive (significant luminal narrowing) atherosclerosis have proven to be strong and independent predictors of MACE, with a three-fold and six-fold increased risk, respectively; as compared to individuals without these findings.[30,31]

Although most data on the prognostic value of CAD in asymptomatic populations are based on the measurement of coronary artery calcification (CAC),[32-34] advanced CAD in PWH, especially in men is characterized by an increase in noncalcified plaque and HRP features such as plaque with low CT attenuation (correlated with necrotic lipid core) and positive remodeling (reflecting eccentric plaque extension) but not calcified plaque (Figures 1 and 2)[6,13] independent of CAD risk factors.[35] These data are consistent with the hypothesis that increased CV morbidity and mortality in the setting of HIV is driven by increased systemic immune activation and inflammation, a phenomenon that leads to an increased influx of inflammatory macrophages, which are more typically found in active non-calcified high risk morphology plaque.[36,37] High risk CAD features are thought to identify individuals at greater risk of plaque rupture and have been associated with MACE in symptomatic HIV-uninfected populations.[38-42] Serial CCTA is the preferred means to providing this assessment of high risk plaque features noninvasively and can detect and quantify changes in noncalcified coronary plaque volume in response to statin therapy[43] (Table I). The potential utility of this technique was demonstrated in a smaller 12 month study among PWH, in which statin therapy reduced noncalcified plaque volume by 19% compared to a 20% increase with placebo, with accompanying decreases in high-risk plaque features and oxLDL.[26,44]

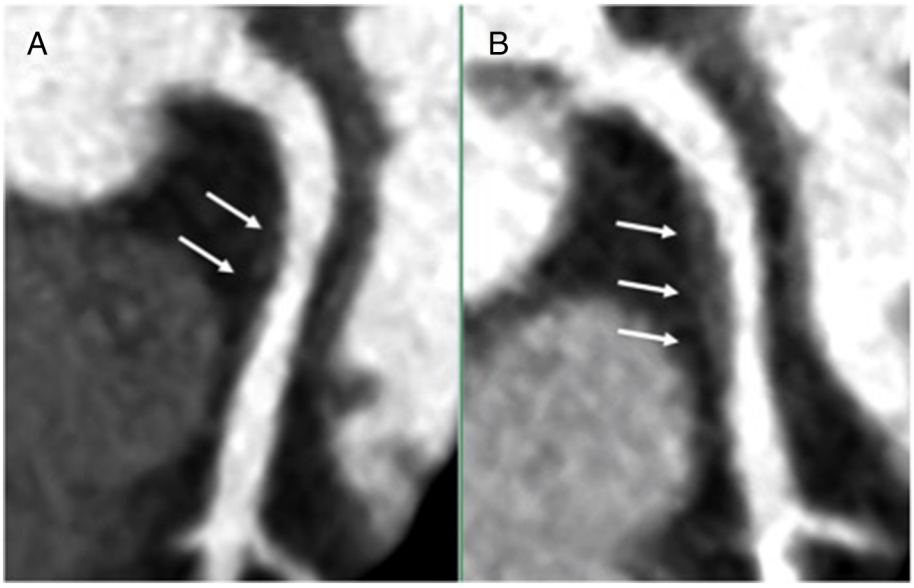
REPRIEVE is a landmark primary prevention trial assessing statin effects on MACE in PWH. Use of serial CCTA, in parallel with assessment of statin effects on key inflammatory and immune pathways in a large subset of participants enrolled in REPRIEVE, will provide a novel opportunity to assess the mechanisms of statin effects on CVD in PWH and provide important context to the findings in REPRIEVE.

Methods

Major objectives

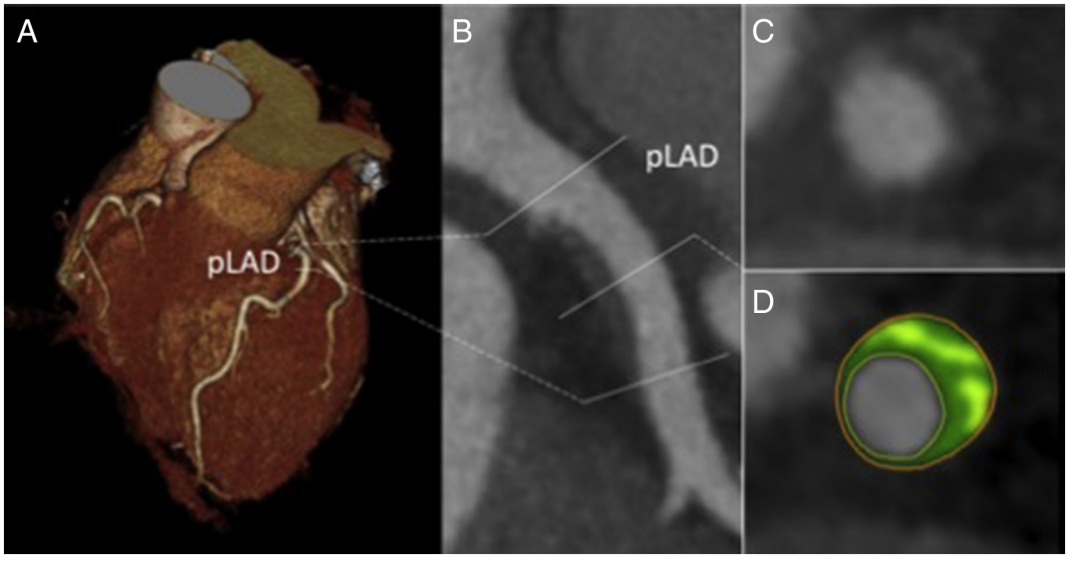
PWH have evidence of high risk plaque and inflammation, often in the absence of increased LDL-C, that may

Figure 1



Curved planar reformats of (A) baseline and (B) 1-year follow-up coronary CTAs in a 58-year-old woman living with HIV. A noncalcified proximal left anterior descending coronary artery plaque (arrows) increased in size while on placebo

Figure 2



Coronary computed tomography angiography (CCTA) (A) 3d volume rendering and (B) curved planar reformat through a proximal left anterior descending plaque. Plaque volume is quantified on the (C) short axis slice through the artery, with the (D) attenuation of plaque voxels categorized as noncalcified or calcified.

contribute to increased CVD morbidity and mortality. The Mechanistic Substudy was designed to formally test whether statin therapy, results in reduction or non-progression of NCP volume, incident NCP, and the presence and extent of HRP among PWH. The addition of a wide panel of biomarkers of immune activation and

Table I. Statin studies using change in coronary plaque volume on Serial CCTA as a measure of therapeutic efficacy

Study	Population	Design	Therapy or Comparison	Main Finding
Inoue K et al. JACC Cardiovasc Imaging. 2010[48]	Non-HIV with CAD, n=32	Prospective Interventional, not randomized	12 months fluvastatin vs refused fluvastatin	Mean plaque volume: Fluvastatin -15.9 mm^3 vs control 4 mm^3 (p=0.01)
Lo J et al. Lancet HIV. 2015[21]	PWH, n=37	Prospective Interventional Randomized Controlled	12 months atorvastatin vs placebo in persons with HIV	Median Δ noncalcified plaque volume: atorvastatin -19% (-8.2 mm3) vs. placebo +20% (+6.7 mm3), p=0.009
Auscher S et al. Atherosclerosis. 2015[49]	HIV-uninfected with acute MI, n=96	Prospective Interventional Randomized Controlled	12 months intensive rosuvastatin vs standard statin	Median Δ plaque volume intensive: +43.5 vs standard: +19.1 mm3, p<0.001
Noguchi T et al. JACC 2015[50]	HIV-uninfected with CAD	Prospective. Interventional, non-randomized	12 months intensive pitavastatin vs propensity matched controls	Mean Δ low attenuation plaque volume: intensive -12.8 vs controls: +8.3 mm3, p=0.004

Abbreviations: *CCTA*, Coronary computed tomography angiography; *CAD*, coronary artery disease; *PWH*, people with HIV; *MI*, myocardial infarction.

inflammation will be key to identifying interactions between atherosclerosis and metabolic pathways including potential upstream mediators of statin effects on plaque and the impact of both mechanisms on outcomes.

The primary objective of the Mechanistic Substudy is to determine the effects of pitavastatin on the morphology and composition of NCP, including the progression of plaque volume and incident NCP and whether these effects are modulated by markers of inflammation and immune activation.

Key secondary objectives are to determine the effects of pitavastatin on the progression and incidence of HRP features, including low attenuation plaque and positive remodeling; to determine the effects of pitavastatin on immune markers, including immune function (CD4, HIV viral load), immune activation (%CD14+CD16+ monocytes, sCD163, sCD14, MCP-1 and T-cell markers), inflammation (Lp-PLA2, hsCRP, IL-6, oxLDL) and coagulation (D-Dimer and tissue factor), as well as traditional CVD risk indices including glucose homeostasis parameters (insulin, glucose and related indices of insulin resistance such as HOMA-IR, HgbA1c); to evaluate the relative contributions of baseline and pitavastatin-induced changes in HIV-specific immune activation and traditional CVD risk factors, including LDL-C, on the presence and progression of coronary plaque and high risk morphological features in HIV; and to assess the relationship of host genetics to study endpoints.

Primary mechanistic hypothesis

The primary hypothesis of the Mechanistic Substudy is that pitavastatin therapy will reduce progression of NCP volume over two years as measured by serial CCTA as compared with placebo in PWH on ART in whom traditional CVD risk is not significantly increased. The mechanisms underlying the effect of statins will include a) reduction in noncalcified coronary atherosclerotic plaque, b) reduction in vulnerability features of noncalcified coronary atherosclerotic plaque, and c) improvement in critical indices of immune activation and inflammation.

Secondary mechanistic hypotheses

The secondary hypothesis of the Mechanistic Substudy is that reduction in LDL-C levels associated with pitavastatin therapy will be predictive of improvement in noncalcified coronary atherosclerotic plaque burden and/or vulnerability features. Additionally, pitavastatin therapy will decrease indices of general inflammation, coagulation, monocyte activation, and arterial inflammation. More specifically, pitavastatin therapy will reduce levels of (a) pro-inflammatory monocyte populations and (b) T-cell activation and exhaustion. The observed changes in immune markers will be associated with changes in morphology and composition of NCP. Lastly, pitavastatin therapy will not have a clinically significant effect on glucose and insulin resistance.

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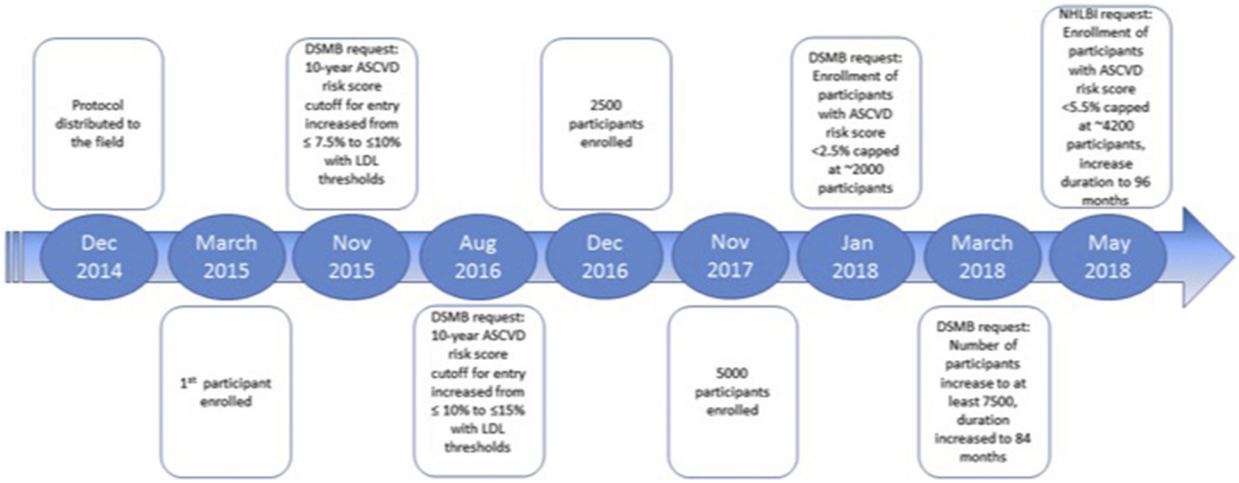
Overall study design

The Mechanistic Substudy is a prospective substudy of approximately 800 participants enrolled in REPRIEVE, a

Figure 3



Timeline of REPRIEVE design changes and amendments

double-blind, randomized, placebo-controlled, multicenter, phase III efficacy trial for primary CVD prevention among HIV-infected patients on stable ART, randomized to 4mg daily pitavastatin vs. matching placebo (Figure 3). Participants co-enrolled in the Mechanistic Substudy will undergo CCTA and blood sampling at baseline and after two years in addition to the procedures for the main REPRIEVE trial, with continued follow up in the main REPRIEVE trial for approximately 48 to 96 months, depending on date of enrollment in the trial.

Site selection and qualification

The Mechanistic Substudy is conducted at 31 US sites out of a total of 116 sites participating in the REPRIEVE trial. Substudy enrollment occurs at the same time as enrollment for the REPRIEVE trial using a separate additional informed consent. Prior to implementation of the Mechanistic Substudy, each site must have the substudy protocol and the informed consent form approved, as appropriate, by their local IRB/EC and any other applicable regulatory entity (RE). Site qualifications for the Mechanistic Substudy include: (a) participation in the REPRIEVE trial, (b) performance by the site radiology or cardiology department of >1000 CT/per year, (c) MD oversight/supervision of CT scans, (d) access to at least 2nd generation 64-slice CT scanner, (e) utilization of level III readers and (f) capability to perform prospective triggering/gating, using low kV and capacity to premedicate participants. Site certification and activation for participation in the Mechanistic Substudy includes mandatory protocol completion requirements such as IRB approval, contract execution, protocol training, and successful submission of two adequate test CCTAs for image quality and radiation safety assessment.

Table II. Eligibility criteria in the Mechanistic Substudy of REPRIEVE (A5333s)

Inclusion criteria
Enrollment in REPRIEVE (A5332)
Willingness to complete procedures required for the study
GFR ≥60 mL/min/1.73m^2 or CrCl ≥60 mL/min, as per REPRIEVE (A5332) within 14 days prior to CCTA
Exclusion criteria
Known allergy to iodinated contrast agent
Currently symptomatic asthma
Allergy to beta blockers
Contraindication to beta blockers (i.e., taking daily asthma medications)
Positive pregnancy test within 24 hours prior to study entry
Any condition that prohibits the individual from completing the CCTA
BMI ≥40 kg/m^2
Cardiac arrhythmia at enrollment precluding CCTA; such as atrial fibrillation with heart rate >80 beats per minute or frequent ectopic beats

Abbreviations: *GFR*, Glomerular filtration rate; *CrCl*, creatinine clearance; *CCTA*, coronary computed tomography angiography; *BMI*, body mass index.

Study population

HIV-infected men and women ≥40 and ≤75 years of age who were eligible and enrolling in the main REPRIEVE trial co-enrolled in the Mechanistic Substudy. Requirements for enrollment in the main REPRIEVE trial include continuous use of ART for at least 6 months prior to study entry with CD4+ T-cell count >100 cells/mm^3. Eligibility for REPRIEVE is further based on ASCVD score and LDL-C (see accompanying manuscript), with

Table III. Anticipated flow cytometry panels to be performed in analyses of PBMCs

Panel	FITC	PE	TxRed-PE	Cy5-PE	Cy5.5-PerCP	Cy7-PE	APC	Alexa 700	Cy7-APC	Pacific Blue	Aqua	QD655
Lymphocytes	CCR7	CD38	CD45RA	CD16	CD4	CD56	CD20	CD3	HLA-DR	PD-1	Live/Dead	CD8
Monocytes #1	-	CCR2	HLA-DR	-	CD163	CD16	CCR5	CD3	CD20	CD14	Live/Dead	CD4
Monocytes #2	CX3CR1	CD169	HLA-DR	-	-	CD16	TF	CD3	CD20	CD14	Live/Dead	-

glomerular filtration rate (GFR) ≥60 mL/min/1.73m^2 or creatinine clearance (CrCl) ≥60 mL/min.

Participant eligibility for the mechanistic Substudy of REPRIEVE

Enrollment into the Mechanistic Substudy occurred concurrently with enrollment and randomization into the main REPRIEVE trial. Eligibility for the Mechanistic Substudy was determined at a screening visit to determine participant's willingness to complete study procedures and to rule out contraindications to CCTA (previous contrast reaction). CCTA was performed within 14 days after randomization in REPRIEVE. Depending on timing of the screening creatinine assessment in relationship to the entry CCTA, an additional serum creatinine was drawn to ensure an evaluation of GFR or CrCl within 14 days prior to CCTA. A GFR ≥60 mL/min/1.73m^{2}or creatinine clearance (CrCl) ≥60 mL/min, calculated using standard equations, is required for the subject to proceed with CCTA both at entry and at month 24. Additional information is collected as part of a participant's enrollment in REPRIEVE on medical history, including CV risk history, physical exam, and medication history (Table II).

Enrollment

Participants are enrolled in the Mechanistic Substudy via the enrollment system at the same time as they are randomized into REPRIEVE. As part of their participation in the main REPRIEVE trial, participants are randomly assigned in a ratio of 1:1 to either 4mg pitavastatin or identical placebo for pitavastatin, 1 pill daily. To ensure balanced treatment allocation in the Mechanistic Substudy, REPRIEVE main study randomization is stratified by planned Mechanistic Substudy participation.

Intervention and subsequent study visits

After completion of the baseline CCTA, participants begin treatment with blinded study medication as per the REPRIEVE protocol. For the Mechanistic Substudy, blood is drawn at the 4 month follow up visit, and CCTA and blood are obtained at the 24 month follow up visit. The 24 month CCTA is conducted under the same protocol, including the same kVp setting as the baseline CCTA with identical blood collection as the baseline visit. Paired scans from baseline and month 24 visits are analyzed side by side with CT readers blinded to treatment assignment and CCTA time point (baseline vs follow-up).

Endpoints

Primary endpoint. The primary outcome measure of the Mechanistic Substudy is change in NCP volume among participants with NCP evidence at entry or incident NCP among subjects without NCP evidence.

Secondary endpoints. Secondary outcome measures include presence and number of HRP features (i.e. low attenuation and positive remodeling on CT); levels of immune, inflammatory and coagulation biomarkers at study entry, month 4, and month 24, and changes from study entry to month 4 and month 24 of immune, inflammatory and coagulation biomarkers; fasting lipid fractions (Total, HDL cholesterol [HDL-C], non-HDL-C, and LDL-C:HDL-C ratio) at study entry, month 4 and month 24; fasting insulin, HgbA1c, and HOMA-IR at study entry, month 4, and month 24 (excluding HgbA1c at month 4; HgbA1c will be available at entry and month 24 only); and time to the first major cardiovascular event. Flow cytometry is performed at Boston College from cryopreserved peripheral blood mononuclear cells (PBMCs) collected at baseline and 24 months, utilizing a standard ACTG preparation procedure to ensure cell viability. This analysis uses gating and analytical procedures to assess activated monocytes, see Table III for anticipated analyses. Immune activation and other inflammatory biomarker measurements are performed at Temple University. Biomarkers measured include: sCD163 (Trillium, now IQ Products), sCD14, monocyte Chemoattractant Protein-1 (MCP-1), Lipoprotein-associated phospholipase A2 (Lp-PLA2), high sensitivity Interleukin-6 (IL-6) (all R & D Systems) and the metabolic marker oxLDL (Mercodia). Samples are analyzed in batches every 6 months by ELISA assays from EDTA-coagulated plasma

Statistical methods

Sample size and power calculations. The target sample size for the Mechanistic Substudy was 800 participants approximately equally distributed between the study arms. This sample size was determined to have high power to detect clinically relevant differences between the two study groups with respect to progression of non-calcified plaque (NCP), defined as progression of baseline NCP among those with plaque at study entry and as rates of incident plaque (among those plaque-free at entry). Specifically, the total sample size of 800 participants will provide 90% power to detect a 6% difference between the study groups in the percent

change in NCP volume over 2 years among those with plaque at entry and 90% power to detect 13 percentage point difference in the probability of plaque development over 2 years. These effect sizes translate to a combined estimated 14 percentage point difference in the probability of NCP progression over two years and are based on the following assumptions: a) 50% of study participants will have evidence of NCP at study entry[5]; b) a SD of 20% for the percent change over 2 years among participants with evidence of plaque at entry; c) an annual rate of incident plaque development of 12% among participants without plaque at entry; d) 15% of participants entering the substudy will not be evaluable for study entry or 2 year NCP volume. Together, the effects of statins acting in these two groups (those with and without evidence of NCP at study entry) will provide for a 7% lower prevalence of NCP after 2-years of statin treatment.

Primary statistical analysis. Among participants with plaque at entry, descriptive statistics for the change and percentage change in NCP volume over 2 years will be provided by treatment group with group comparisons made with stratified t-test. Among those without NCP at entry, the prevalence of incident NCP over 2 years will be compared with stratified chi-squared test. To assess the mechanistic study population as a whole, participants will be classified as progressors (any progression/increase in NCP volume OR incident NCP) or non-progressors (no progression in NCP volume OR no incident NCP); the probability of progression over two years will be compared by treatment group using a stratified chi-squared test. All treatment group comparisons will be performed by intention to treat using a 5% type error. Unless otherwise noted, analyses will be performed by subgroups defined according to the presence of NCP at study entry.

Secondary and sensitivity analyses. *Statin effects on coronary plaque morphology*. The statin effect on HRP features, including low HU attenuation and positive remodeling, will be assessed by comparing differences in the 2-year prevalence of high-risk plaque morphology features between treatment groups using chi-squared (or Fisher's exact) tests as appropriate; these analyses will be performed overall and by subgroups defined by the presence of NCP at study entry. Exploratory analysis will be performed for additional high-risk plaque features that have been described in CCTA and intravascular ultrasound (IVUS) studies including the napkin-ring sign, minimal luminal area, plaque burden, and segments with NCP. The analytic approach will be similar as described above.

Statin effects on blood biomarkers. Statin effects on the distributions of blood biomarkers belonging to distinct pathways (i.e., monocyte activation, generalized inflammation, and coagulation) will be assessed via treatment group comparisons of these respective markers via t-tests; modification of statin effect on these markers by HIV-1 and traditional risk factors (including sex, age, screening CD4, duration of suppressive ART, and presence of NCP at study entry) will be assessed. Since the hypothesized mechanism is that sustained high levels of immune activation and inflammation precede and contribute to progression of NCP volume and HRP features, these analyses will relate short-term changes in these biomarkers (over 4 months) to longer term changes in NCP volume and morphology after two years.

LDL-C and blood biomarkers as mediators for plaque progression. In the event that both statin effects on NCP progression and biomarker changes are apparent, the association between changes in LDL-C and these biomarkers and NCP progression will be examined using graphical techniques and normal errors and logistic regression (for the subpopulations with and without NCP at entry respectively. A mediating effect of these biological factors will be evaluated by examination of changes in the estimated statin effect on NCP after adjustment for these biological factors.

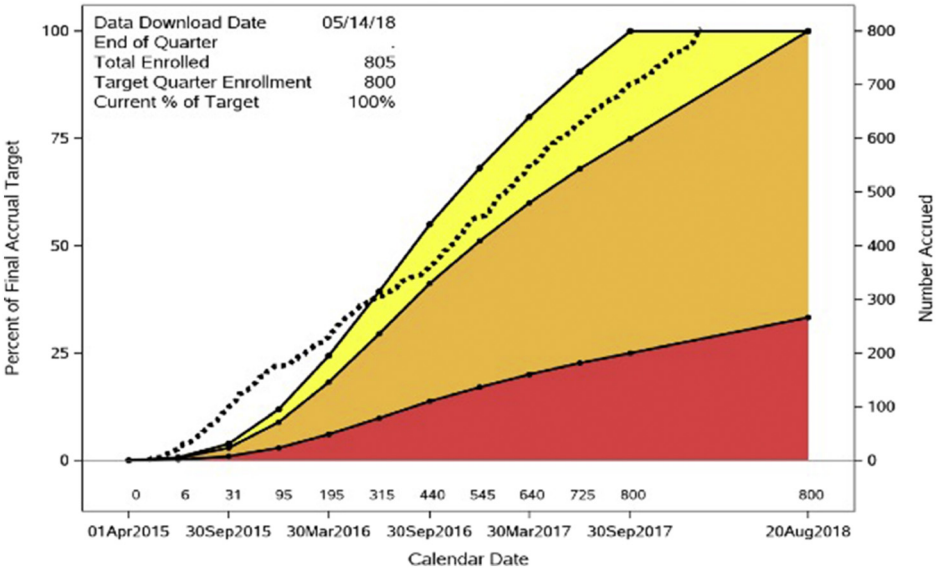
Plaque Progression and MACE. These hypothesis generating analyses will assess the association of baseline CAD characteristics including NCP volume and HRP, 2-year changes in NCP volume and 6-month and 2-year changes blood biomarkers and the hazard of MACE to provide valuable insights to the mechanisms of CVD in HIV and the role of statins in reducing CVD burden in this population. Those biomarkers with the strongest mediating effect on plaque progression will then be measured in the entire REPRIEVE cohort to determine their association with MACE.

Organization and human Studies approval

The Mechanistic Substudy is conducted under the supervision of an Executive Committee (EC). The DCC coordinates all procedures associated with the Mechanistic Substudy, and performs all aspects of protocol monitoring; protocol development; and site selection, evaluation, and performance monitoring; guides image acquisition; and is responsible for receiving, tracking, and archiving CCTA images that are obtained during the study. Quality control (QC) checks for image quality and completeness of all CCTAs are also performed by the DCC. Radiation safety is supervised by the DCC. Frontier Science Foundation is the REPRIEVE data management center and provides and maintains infrastructure for data collection and supervises visit tracking for the substudy as well as the main REPRIEVE trial, AE reporting, and specimen handling and shipping.

The NHLBI appointed an independent Data and Safety Monitoring Board (DSMB) to monitor participant safety and provide recommendations regarding termination, continuation or modification the study protocol as necessary. Data from the Mechanistic Substudy is reviewed in parallel with data from the main REPRIEVE trial by REPRIEVE DSMB every 6 months. The Partners Health Care Institutional Review Board approved the

Figure 4



Mechanistic Substudy accrual figure. Dotted line shows actual accrual progress. Colored bands denote predefined NIH target enrollment zones: yellow (100%-75%), orange (75%-25%), red (<25%).

protocol and provides oversight of central activities of the Coordinating Centers, including implementation of design changes and overall trial structure.

Results

As of February 2018, the Mechanistic Substudy completed enrollment of 805 participants at 31 US-ACTG sites (Figure 4). Work has begun to analyze paired scans as well as inflammatory biomarkers from baseline and 2 years as participants complete the substudy.

Discussion

Statin therapy lowers CVD events by 30% in classic primary and secondary prevention settings.[45,46] However, the mechanisms of such effects remain unknown. This substudy of REPRIEVE was designed to assess potential mechanisms of statins on CVD in PWH, by determining serial plaque measurements, and key inflammatory and immune biomarkers in a large subset of patients enrolled in REPRIEVE. The Mechanistic Substudy will add significantly to the understanding of the results of REPRIEVE.

IVUS studies in non-HIV infected individuals have shown a variable association between statin therapy and change in atherosclerosis with changes ranging from minor regression (-2.3%) to minor progression (+1.5%)[47] despite uniformly substantial lowering in LDL-C. The use of CCTA provides substantial benefits over traditional IVUS techniques for the assessment of CAD progression, including the ability to assess changes in a more generalizable manner across the entire coronary artery tree in a noninvasive fashion.

In addition, recent research has focused on the pleiotropic anti-inflammatory effects of statins as an additional explanation for their observed benefits. Indeed, JUPITER[48] and CANTOS[49] have tested the hypothesis that that reduction in inflammation may contribute to reducing CVD event rates. Unfortunately, none of these studies included imaging of CAD and hence a mechanistic explanation for the observed benefits, specifically whether a change in high-risk features of CAD such as NCP or HRP may have been causally responsible, could not be provided. In fact, to date, no such large trial combining coronary architecture with immune/inflammatory signals has been conducted either in people with or without HIV.

The Mechanistic Substudy of REPRIEVE will assess the mechanisms of statins within the context of a large randomized primary prevention trial of statin therapy in PWH with low to moderate traditional cardiovascular risk. Thus, this substudy offers a unique opportunity to study statin effects in a population whom traditional risks are not significantly increased, and in which inflammation and immune activation is thought to contribute to high risk plaque and potential plaque rupture. The Mechanistic Substudy of REPRIEVE will take advantage of the main study randomization, and rigorously determine statin benefits in this population utilizing serial CCTA and deep phenotyping of inflammatory and activation markers.

The study was launched in 2015 and has completed enrollment and baseline evaluations of 805 participants, utilizing 31 US sites, most of which are participating in the NIH-supported AIDS Clinical Trials Group (ACTG), with access to high quality CCTA and established biomarker preparation techniques, and is based on a highly successful, and novel collaboration among multiple stakeholders, including NHLBI, NIAID, OAR, and the ACTG.

An important goal of the REPRIEVE Mechanistic Substudy is to provide key scientific insights into how statin therapy affects CCTA-based plaque morphology and whether any plaque changes are predated by and related to statin-induced changes in immune activation and inflammation among PWH. In addition, we will determine whether the potential effects of statins on stabilization of atherosclerotic plaque—rendering plaques less likely to rupture and cause acute myocardial infarction—are mediated primarily through lipid-lowering effects or through immunomodulatory effects, and whether plaque stabilization is related to baseline traditional risk factors.

This trial was designed to demonstrate the effect of statin therapy on CAD among PWH in whom traditional risk factors are often not increased. This study has relevance to other disease populations with an increased inflammatory potential such as rheumatoid arthritis.[50] Thus, PWH represent an important population in which to test whether CVD-protective effects of statins is mediated through pleotropic effects on immune activation and inflammation[9,37,51] as well as lipid lowering.

REPRIEVE and its Mechanistic Substudy will help to provide information to guide primary CVD prevention for PWH, a population for whom appropriate algorithms for predicting CVD events have not been fully developed and among whom risk stratification is challenging.[52-54] REPRIEVE relies on a recently developed algorithm employing assessment of 10-year ASCVD risk score via the 2013 ACC/AHA risk calculator, with paradigm modifications to permit enrollment of higher-risk participants with lower LDL-C levels. Data on the utility of the 10-year ASCVD risk score are retrospective, and no study has assessed this algorithm prospectively.[53,54] We have shown that CAD, as detected by CCTA, effectively reclassifies ASCVD risk in individuals without HIV,[31,55] and it will be important to assess how this new risk score relates to plaque morphology and ultimately MACE events in the HIV population.

Given that substudy enrolment was complete before changes in the ASCVD risk score requirements were made, proportionally more participants with lower ASCVD risk scores are expected in the substudy population than in a comparable US-based population in the main study. Given the timing of the enrollment restrictions relative to full enrolment of the parent study, this shift is expected to be modest.

Summary and significance

To our knowledge, the REPRIEVE Mechanistic Substudy represents the first large substudy of a primary CVD prevention trial to specifically assess statin mechanisms in any population. We will utilize state of the art CCTA assessment on high-risk plaque paired with detailed immunophenotyping to provide much needed insights into whether the benefits of statins on CVD prevention in PWH can be explained by modifications in coronary plaque as well as whether these changes are mediated by changes in key immune and inflammatory indices, and how both are related to adverse outcomes.

Author contributions

All authors have read and approved this manuscript.

Disclaimer

The views expressed in this article are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institute of Allergy and Infectious Diseases; National Institutes of Health; or the United States Department of Health and Human Services.

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