Effects of Pitavastatin on Coronary Artery Disease and Inflammatory Biomarkers: Mechanistic Substudy of REPRIEVE (A5333S)

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This is REPRIEVE (A5333S) Primary SAP Version 1.1 with names of authors and investigators removed. Other redactions for public release are as indicated throughout the document.

1 Background

A5333s is an optional mechanistic substudy of REPRIEVE (A5332). Approximately 800 people with HIV (PWH) who are enrolled in REPRIEVE (A5332) were targeted for Mechanistic Substudy of REPRIEVE (A5333s) enrollment.

The overarching aim of the Mechanistic Substudy of REPRIEVE (A5333s) is to better understand the modulation of critical features of coronary plaque morphology with statin therapy, including the progression of noncalcified coronary atherosclerotic plaque (NCP) volume in HIV and the biological factors mediating these effects during statin treatment in HIV. The Substudy will determine potential statin effects to halt progression of non-calcified atherosclerotic plaque and to stabilize morphologic features of plaque vulnerability. Moreover, the study will identify biological factors mediating these changes – be it lipid parameters, such as LDL cholesterol, or markers of inflammation and immune activation. Finally, the study will evaluate whether presence and morphology of subclinical atherosclerotic plaque at baseline relates to CVD events independently of traditional CVD risk factors and markers of HIV-specific immune activation.

This is the Primary Statistical Analysis Plan for the Mechanistic Substudy of REPRIEVE. It expands on details of the statistical analysis plan that were defined in Appendix II of the REPRIEVE (A5332) protocol document. Major changes from the details defined in the protocol are outlined in the version history table below. An overview of the Substudy design is provided below; details of the substudy sample size considerations are in Appendix I, copied verbatim from the protocol.

In preparation for public release of the document with publication of the data, preliminary substudy data and published work from other groups that were used as sample tables have been redacted.

Version History

Version	Changes Made	Date Finalized
(Protocol)	Original Version (Version 2.0)	Dec 19, 2014
1.0	Clarification that primary outcomes are related to noncalcified plaque; additional secondary plaque outcomes added. HIV-1 RNA and CD4 moved from secondary outcomes to risk factors. HgA1C removed throughout (no longer being tested).	Mar 20, 2023
1.1	For Published release of the SAP … Section 7: Sample tables of published work from other groups redacted. Appendix II: Draft consort based on early preliminary data removed.	October 4, 2023

1.1 A5333S Design Overview

Enrollment into A5333s occurred concurrently with enrollment and randomization into REPRIEVE (A5332) at select US ACTG sites participating in REPRIEVE (A5332). To ensure balance in treatment assignment in the Substudy, randomization in REPRIEVE (A5332) was stratified by anticipated Substudy participation.

Substudy participants were followed for 2 years with serial coronary computed tomography angiography (CCTA) performed at study entry and 2 years. Fasting blood samples for assessment of lipids and select soluble biomarkers were drawn at entry, month 4, and year 2; PBMCs for assessment of cellular markers of immune function were drawn at entry and year 2. The Substudy also included a 2-year assessment of REAP (diet and physical activity) and DASI (physical function), as well as quality of life assessment using the SF-36 at entry and year 2.

Entry blood draws and CCTA were scheduled to occur within 14 days after randomization and prior to the start of randomized treatment. Month 4 evaluations were to be performed ± 21 days and year 2 evaluations were to be performed ±28 days. In light of scheduling concerns and to maximize the collection of follow-up CT scans, the window for year 2 data collection was extended to up to 30 months after the entry CT.

1.2 A5333S Hypotheses

1.2.1 Primary Hypothesis

Statin therapy will reduce progression of noncalcified coronary atherosclerotic plaque (NCP) volume over two years as measured by CCTA, as compared with placebo in HIV-infected persons at low to moderate traditional ASCVD risk.

1.2.2 Secondary Hypotheses

- Decreases in LDL cholesterol levels associated with statin therapy will predict improvement in NCP burden and/or plaque vulnerability features.
- Statin therapy will reduce indices of general inflammation, coagulation, monocyte activation, and arterial inflammation.
- Statin therapy will reduce levels of pro-inflammatory monocyte populations.
- Statin therapy will reduce levels of T-cell activation and exhaustion.
- Changes in levels of immune activation and inflammatory markers will be associated with changes in morphology and composition of NCP.
- Statin therapy will not have a clinically significant effect on glucose and insulin resistance.

1.3 A5333S Timeline

- The Substudy opened to enrollment on April 14, 2015; first enrollment occurred on May 6, 2015.
- Substudy enrollment closed on February 6, 2018.
- A memo was sent to Substudy sites on March 12, 2020, requesting completion of all follow-up CT scans by June 1, 2020. The Substudy did not formally close to follow-up at this time, pending a possible extension and additional follow-up CT scan.

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2 Objectives and Estimands

2.1 Primary Objective

To determine the effects of pitavastatin on the morphology and composition of coronary atherosclerotic plaque, (including the progression of noncalcified plaque (NCP) volume), and whether these effects are modulated by markers of inflammation and immune activation.

Primary Estimand	
Estimand description	*An estimand defines the target of estimation for a particular trial objective (i.e. "what is to be estimated"). This combines all the estimand elements outlined below into a single, succinct sentence.*
Treatment	Treatment groups
Pitavastatin 4mg per day	Pitavastatin 4mg per day or placebo
Target population	Analysis set
PWH on stable ART with low to moderate cardiovascular risk and no known current indication for statin treatment.	All randomized participants with a REPRIEVE (A5332) entry visit. Participants who were consented but never returned to the clinic for their REPRIEVE (A5332) entry visit (regardless of whether the participant was formally randomized) will be considered inadvertent enrollments and excluded.
Variable(s)	Outcome measure(s)
Change in noncalcified plaque volume (NCP) *Change in volume of plaque and calcified plaque will be examined as secondary variables.*	*See Section 3 for more detailed outcome descriptions* • Absolute change in NCP volume from entry to year 2. • NCP progression defined as any increase (>0) in NCP volume or incident NCP for those without NCP at entry (based on qualitative read) For sensitivity analyses, • Qualitative assessment of at least one segment with NCP on the 2 year CT. • Quantitative NCP volume at 2 year CT
Handling of intercurrent events	Handling of missing data
Failure to start statin: Included per randomized treatment (treatment policy) Change in statin strategy: Included per randomized treatment (treatment policy) *Includes discontinuation or change of statin* Evidence of critical stenosis at treatment start: Included per randomized strategy (treatment strategy) Interval stent placement or bypass*: Included per randomized strategy (treatment policy strategy) Death prior to Year 2 scan: Treated as missing data (hypothetical policy)	Missing data due to missed visits or unreadable scans will be assumed missing completely at random and ignored i.e., the primary analysis will be a complete case analysis including participants with pair baseline and year 2 scans. Sensitivity analyses will include multiple imputation for missing data.
Population-level summary measure	Analysis approach
Mean change NCP volume over 2 years	Linear regression (normal errors assumption). In the event of extreme violation of normality, outcomes will be log transformed (giving a percentage change interpretation of the treatment effect). Modeling will adjust for CD4 and sex (stratification factors), and for baseline NCP volume.
** Stented or bypassed coronary artery segments are not considered on the entry and year 2 scan as part of the read protocol for plaque volume.*	

2.2 Secondary Objectives

- To determine the effects of pitavastatin on the progression of high risk plaque features including low attenuation plaque and positive remodeling.
- To determine the effects of pitavastatin on detailed markers of immune function including, immune activation (%CD14+CD16+ monocytes, sCD163, sCD14, MCP-1 and T-cell markers), inflammation (Lp-PLA2, hsCRP, hs troponin, oxLDL IL-6), coagulation (D-Dimer) and traditional CVD risk indices

including detailed parameters of glucose homeostasis (insulin, glucose and related indices of insulin resistance such as HOMA-IR).

- To determine the relative contributions of baseline and pitavastatin induced changes in HIV-specific immune activation and traditional risk factors, including LDL, on the presence and progression of coronary plaque and high-risk morphological features in HIV.

3 Outcome Measures

3.1 Analysis Windows for Evaluations

Evaluations drawn within the windows of evaluation defined below will be included in outcome measures; evaluations outside these windows will be excluded. It is noted that analysis windows for month 24 evaluations were widened from those originally planned to accommodate scheduling complications due to COVID restrictions.

Evaluation	Entry*	Month 4	Year 2 (Month 24)
CT scan	Any date ≤ (First dose+14 days)	-	Target date – 6 months (180 days) to Target date + 12 months (360 days) i.e. Month 18 to Month 36
Fasting lipids	Any date ≤ First dose	Target date ± 60 days	
Soluble biomarkers	Any date ≤ First dose	Target date ± 60 days	
Flow cytometry	Any date ≤ First dose	-	
Questionnaires	Any date ≤ First dose	-	
** For participants who never start treatment, evaluations ≤ 30-days after A5332 entry.* *Target date = A5332 entry date + (Month*30)*			

3.2 Primary, Secondary, and Associated Supportive Outcome Measures

The primary variable of interest for the Substudy is the morphology and composition of noncalcified coronary atherosclerotic plaque. The following NCP outcome measures will be described and compared by treatment group. Corresponding outcomes at entry will be described when appropriate. For the primary analysis, a threshold of <350 HU will be used to define non-calcified plaque.

3.2.1 Change NCP volume over 2 years (Primary)

- Continuous outcome:
  - Absolute change: NCP volume at 2 years – NCP volume at entry
  - Percentage change: (NCP volume at 2 years – NCP volume at entry) / NCP volume at entry (Supportive)
- Based on quantitative read whenever available (including for participants with no evidence of NCP based on the corresponding qualitative read).
  - Participants without a quantitative read and no evidence of NCP based on the corresponding qualitative read will be assigned a value of zero for both absolute and percentage change.

3.2.2 Progression of NCP over 2 years (Primary)

- Binary outcome (Yes, No)
- Based on both quantitative and qualitative reads
  - Among participants without evidence of NCP at entry on the qualitative read, evidence of NCP at 2 years.
  - Among participants with evidence of NCP at entry on qualitative read, >0% increase in NCP volume
  - Participants with discordant outcomes based on qualitative and quantitative reads, progression will be defined by the quantitative read.

3.2.3 NCP on the 2 year CT (supportive)

- Binary outcome (Yes, No)
- Based on qualitative read
- Outcome is defined regardless of availability of an entry scan

3.2.4 NCP volume at 2 years (supportive)

- Continuous outcome
- Based on quantitative read whenever available (including for participants with no evidence of NCP on the corresponding qualitative read).
  - Participants without a quantitative read and no evidence of NCP on the corresponding qualitative read will be assigned a value of zero.
- Outcome is defined regardless of availability of an entry scan

3.2.5 Total plaque volume

- Analogous outcomes to those in Section 3.2.1-3.2.4 but defined based on total plaque volume (rather than NCP)
- Outcomes will be described for the cohort at entry and year 2, with formal comparisons by treatment group.

3.2.6 Change in relative composition of coronary plaque

- Continuous outcome
- Calculated as the ratio of NCP to overall plaque volume

3.3 Exploratory Plaque Outcomes

The following are additional CT-related outcomes that will be described for the cohort at entry and year 2, as appropriate. *Note: Not all of these analyses will be included in the primary manuscript; but will be included in the packet of primary analyses for completeness.*

3.3.1 Number of segments with plaque

- Ordinal outcome: (0, 1-2, ≥3)
- Based on qualitative read

3.3.2 Number of segments with noncalcified portion

- Ordinal outcome: (0, 1-2, ≥3)
- Based on qualitative read

3.3.3 Presence of vulnerable plaque features

- Five binary (Yes, No) outcomes based on the qualitative read
- Presence of any following vulnerable features (main outcome) and the prevalence of each feature descriptively (four outcomes)
  - Positive remodeling
  - Low attenuation plaque
  - Positive remodeling and low attenuation plaque
  - Napkin ring sign

3.3.4 Alternative definition of noncalcified plaque volume, using a threshold of <130 HU

- Continuous outcome:
  - Absolute change: NCP volume at 2 years – NCP volume at entry
  - Percentage change: (NCP volume at 2 years – NCP volume at entry) / NCP volume at entry (Supportive)
- Based on quantitative read whenever available (including for participants with no evidence of NCP based on the corresponding qualitative read).
  - Participants without a quantitative read and no evidence of NCP based on the corresponding qualitative read will be assigned a value of zero for both absolute and percentage change.

3.3.5 Low attenuation plaque volume (<30 HU)

- Continuous outcome
- Based on quantitative read whenever available.

- Participants without a quantitative read and no evidence of low attenuation plaque on the corresponding qualitative read will be assigned a value of zero

3.3.6 Stenosis

- Three outcomes based on qualitative read
- Presence of CAD or stenosis >0%
  - Binary (Yes, No)
- CAD severity
  - Ordinal (Minimal, Mild, Moderate, Severe)
    - No stenosis
    - Minimal CAD, stenosis 1-24%
    - Mild CAD, stenosis 25%-49%
    - Moderate CAD, stenosis 50%-69%
    - Severe CAD, stenosis ≥70% or ≥50% left main
- Presence of stenosis ≥50%
  - Binary (Yes, No)

3.3.7 Progression of NCP over 2 years

- Ordinal outcome (Progression, Stable, Regression)
- Based on both quantitative and qualitative reads
  - Progression: *Defined per 3.1.4*
  - Regression:
    - Based on quantitative read: >0% decrease in NCP volume over two years.
  - Stable:
    - Based on qualitative read: No evidence of NCP at entry and 2 years.
    - Based on quantitative read: Percentage change in NCP volume between -2% and +2% (including exactly -2% and +2%).
  - For participants with discordant outcomes based on qualitative and quantitative reads, progression will be defined by on the quantitative read.

3.3.8 Coronary artery calcium (CAC), Leaman, and Segment Involvement (SIS) scores

- CAC: Continuous and ordinal (0, 1-10, 11-100, 100-300, >300
- Leaman: Ordinal (0, >0-5, >=5)
- SIS: Ordinal (0, 1, 2, 3, 4, 5+)

3.4 Markers of Immune Function

Levels at entry, 4 months and 2 years, and changes from study entry to 4 months and 2 years (unless stated otherwise).

- Soluble markers of monocyte activation
  - sCD163, sCD14, MCP-1
- Monocyte populations (entry and 2 years only)
  - %CD14+CD16+ (inflammatory/intermediate) monocytes
    - Classical and non-classical subsets presented descriptively
- Markers of T-cell activation and T-cell exhaustion (entry and Year 2 only)
  - CD38+DR+ on CD4+ and CD8+
- Markers of inflammation:
  - Lp-PLA2, hsCRP, IL6, hs troponin, oxLDL
- Markers of coagulation
  - D-Dimer

Future analyses will interrogate the broader spectrum of immune phenotyping that has been performed collected PBMCs.

3.5 Markers of Glucose Homeostasis

Levels at entry, 4 months and 2 years, and changes from study entry to 4 months and 2 years.

- Fasting insulin, glucose, and HOMA-IR

3.6 Descriptive Outcomes

The following REPRIEVE outcome measures will be described by treatment group for the substudy population but since statin effects for these outcomes will be determined based on the full REPRIEVE cohort, formal treatment comparisons will not be performed.

3.6.1 Time to the first major cardiovascular event

- As defined in the REPRIEVE Primary Statistical Analysis Plan

3.6.2 Fasting lipid fractions

- Total, LDL, non-HDL, and HDL cholesterol, and LDL:HDL ratio at study entry, month 4, and 2 years
  - LDL will be reported as calculated LDL if triglycerides ≤400 mg/d; direct LDL if triglycerides >400 mg/dL and <500 mg/dL; LDL will be considered missing if triglycerides ≥500 mg/dL

4 Analysis Sets and Handling of Missing Data

4.1 Primary (Intent-to-treat)

The primary ITT sample will include all registered substudy participants who completed their REPRIEVE (A5332) entry visit. Participants registered to the substudy who failed to complete a REPRIEVE (A5332) entry visit will be considered inadvertent enrollments and excluded from all data summaries and analyses.

The primary analyses will assume all missing data are missing completely at random and be ignored. The intercurrent event of participant death during the 2 year follow-up is expected to be rare and will be handled using a hypothetical strategy – that is, outcomes unavailable due to death will be treated as missing data. Baseline and Year 2 outcomes will be described among all participants with data available at the respective time-point; analyses of change will include only participants with paired scans.

Sensitivity analyses will use multiple imputation with randomization stratification factors (sex and CD4 count) plus ASCVD risk score and BMI used to develop the model.

4.2 Per Protocol (Treated)

All analyses will be repeated within a per protocol sample which will be limited to participants who completed 24–months study follow-up on randomized treatment.

Unless the primary sensitivity analyses demonstrate a concern for informative missingness, per protocol analyses will be limited to participants with paired entry and year 2 scans available with no additional sensitivity analyses performed. Cardiovascular risk factors associated with dropping out of the per protocol analysis will be examined.

5 Administrative Details

Limited to participants in the ITT analysis set.

5.1 Participant Enrollment

Figure: Enrollment over time

- NHLBI enrollment figure

Table: Enrollment by site and sex

- Including details of all participants enrolled at substudy site

5.2 Participant Study and Treatment Disposition

Table: Participant status

			Treatment Group	
		Total (N=XX)	**Pitavastatin (N=XX)**	**Placebo (N=XX)**
Enrolled		**XX (xx%)**	**XX (xx%)**	**XX(xx%)**
Completed 24 mo follow-up		**XX (xx%)**	**XX (xx%)**	**XX(xx%)**
Timing of final visit (months from enrollment))	Median (P10-P90)			
	Min-Max			
Did not complete substudy		**XX (xx%)**	**XX (xx%)**	**XX(xx%)**
Reason for Discontinuation	*Reasons from A5333S RP0002*	XX	XX	XX
	….	XX	XX	XX
Timing of discontinuation	Before 4 month visit*	XX (xx%)	XX (xx%)	XX(xx%)
	Between 4 and 24 month visits	XX (xx%)	XX (xx%)	XX(xx%)

Figure: Time to substudy discontinuation

- KM plot by treatment group

Table: Treatment disposition

			Treatment Group	
		Total (N=XX)	**Pitavastatin (N=XX)**	**Placebo (N=XX)**
Days to start of study treatment*	N	XX	XX	XX
	Median (P10-P90)	XX (XX-XX)	XX (XX-XX)	XX (XX-XX)
	Min-Max	0-X,XXX	0-X,XXX	0-X,XXX
	# missing	XX	XX	XX
	0-2	XX (XX%)	XX (XX%)	XX (XX%)
	3-14	XX (XX%)	XX (XX%)	XX (XX%)
	>14	XX (XX%)	XX (XX%)	XX (XX%)
	Will never start	XX (XX%)	XX (XX%)	XX (XX%)
Timing of treatment discontinuation	Before 4 month visit*	XX (XX%)	XX (XX%)	XX (XX%)
	Between 4 and 24 month visits	XX (XX%)	XX (XX%)	XX (XX%)
	On or after the 24 month visit	XX (XX%)	XX (XX%)	XX (XX%)
Reasons for discontinuation	*Reasons from A5332 RP0003*	XX (XX%)	XX (XX%)	XX (XX%)
	…	XX (XX%)	XX (XX%)	XX (XX%)

** Includes participants who never started treatment*

Figure: Time to treatment discontinuation

- KM plot by treatment group

5.3 CT Scan Availability

Table: CT scan availability

Table denominator is the ITT analysis set; table will be repeated in the per protocol analysis set.

			Treatment Group	
		Total (N=XX)	**Pitavastatin (N=XX)**	**Placebo (N=XX)**
Evaluable Entry CT scan	Qualitative read	XX (XX%)	XX (XX%)	XX (XX%)
	Quantitative read	XX (XX%)	XX (XX%)	XX (XX%)
	Paired reads	XX (XX%)	XX (XX%)	XX (XX%)
No Entry CT scan scan		XX (XX%)	XX (XX%)	XX (XX%)

			Treatment Group	
		Total (N=XX)	**Pitavastatin (N=XX)**	**Placebo (N=XX)**
Reason no entry CT scan	Out of window	XX	XX	XX
	Missed visit	XX	XX	XX
	.<other reasons>	XX	XX	XX
	Image quality	XX	XX	XX
Evaluable Year 2 CT scan	Paired reads	XX (XX%)	XX (XX%)	XX (XX%)
	Qualitative read	XX (XX%)	XX (XX%)	XX (XX%)
	Quantitative read	XX (XX%)	XX (XX%)	XX (XX%)
No Year 2 CT scan		XX (XX%)	XX (XX%)	XX (XX%)
Reason no scan	Out of window	XX	XX	XX
	Premature discontinuation	XX	XX	XX
	Missed visit	XX	XX	XX
	<other reasons>	XX	XX	XX
	Image quality	XX	XX	XX
Evaluable paired scans	Qualitative read	XX (XX%)	XX (XX%)	XX (XX%)
	Quantitative read	XX (XX%)	XX (XX%)	XX (XX%)
	Paired reads	XX (XX%)	XX (XX%)	XX (XX%)

Possible <Other reasons> based on the scan tracking form might include: Refusal, Unable to place IV or other technical issue, Renal insufficiency, Contrast allergy, BMI >40.

5.4 Consort

See Appendix II for draft Consort based on scan reads. This will be expanded on to incorporate details of treatment status and analysis windows.

- Registered
  - Did not complete A5332 entry visit
- Within the ITT sample and by treatment group, Cross-tabulation of
  - Scan availability by treatment status
    - Baseline CTA completed (in versus outside of window)
    - Diagnostics baseline CTA
    - Completed follow-up CTA (in versus outside of window)
    - Diagnostic baseline and follow-up CTA
  - Treatment status
    - Completed treatment
    - Did not start treatment
    - Early treatment discontinuation

5.5 Biomarker Availability

Table: Data availability Like the table below but scaled down out of window visits/samples.

				of Expected Subjects		Total Biomarkers	
Visit	*[Visit windows]*		**Subjects Expected**	**Observed Visits**	**Biomarkers available**	**for day**	**for day & Baseline**
Entry	***TD-180***	***TD+1***	**XX**	**XX (XX%)**	**XX (XX%)**	**XX**	
Entry +	*TD+2d*	*TD+59d*	XX	XX [XX%]	XX [XX%]		
Month 4-	*TD-60d*	*TD-30d*	XX	XX [XX%]	XX [XX%]		
Month 4	***TD±30d***		**XX**	**XX (XX%)**	**XX (XX%)**	**XX**	**XX**
Month 4+	*TD+30d*	*TD+60d*	XX	XX [XX%]	XX [XX%]		
Month 12			XX	XX	XX		
Month 24-	*TD-180d*	*TD-30d*	XX	XX [XX%]	XX [XX%]		
Month 24	***TD±30d***		**XX**	**XX (XX%)**	**XX (XX%)**		
Month 24+	*TD+30d*	*TD+360d*	XX	XX [XX%]	XX [XX%]		

			of Expected Subjects		Total Biomarkers	
Visit	*[Visit windows]*	**Subjects Expected**	**Observed Visits**	**Biomarkers available**	**for day**	**for day & Baseline**
Month 36+		XX	XX	XX		
TD - Target date						

6 Participant Characteristics and Other Risk Factors

See JAMA Open Table 1. Statistics by treatment group as described will be given in the study report; the summaries will likely be streamlined for the manuscript.

The following defined at study entry only unless stated otherwise. The table denominator would be the ITT sample. A second table of the same information will be included for those with paired scans.

- Demographic and Behavioral characteristics
  - Age (years): Mean (SD) and percent by group (40-49; 50-59; 60+)
  - Sex: Percent women
  - Gender identity: Percent by group (Cisgender, Transgender spectrum, Not reported)
  - Race: Percent by group (White, Black or African American, Asian, Other)
  - Ethnicity: Percent by group (Hispanic or Latino, Not Hispanic or Latino, Unknown)
  - Other substance use (cocaine, heroin, methamphetamine)
- ASCVD Risk Score at REPRIEVE Entry
  - ASCVD risk score (%): Median (Q1, Q3) and percent by group (<2.5, 2.5-<5, 5-<10, 10+)
  - Smoking status: Percent by group (Current, Former, Never)
  - Use of antihypertensive medication: Percent yes
  - Systolic BP of those not on antihypertensive medication: Median (Q1-Q3) (P10, P90)
  - Total cholesterol (mg/dL) : Median (Q1-Q3) (P10, P90)
  - HDL-C (mg/dL) : Median (Q1-Q3) (P10, P90)
  - LDL calculated (mg/dL) : Median (Q1-Q3) (P10, P90)
  - Triglycerides (mg/dL) : Median (Q1-Q3) (P10, P90)
  - Family history of premature CVD: Percent yes
- Other Cardiovascular / metabolic characteristics at REPRIEVE Entry
  - BMI: (kg/m2) Mean (SD) and percent by group (<24.9, 25-29.9, 30+)
  - Prior statin use: Percent yes
    - Based on site report at entry
  - Diabetes: Percent yes
  - Use of anti-diabetic therapy: Percent yes
  - Use of ACE/ARBs: Percent yes
  - Use of anti-platelets/anticoagulants: Percent yes
  - Use of non-statin lipid lowering agents Percent yes
- Cardiovascular / metabolic characteristics at Year 2
  - BMI: (kg/m2) Mean (SD) and percent by group (<24.9, 25-29.9, 30+)
  - Prior statin use: Percent yes
    - Based on site report at entry
  - Diabetes: Percent yes
  - Use of anti-diabetic therapy: Percent yes; percent adding medication after baseline
  - Use of ACE/ARBs: Percent yes; percent adding medication after baseline
  - Use of anti-platelets/anticoagulants: Percent yes; percent adding medication after baseline
  - Use of non-statin lipid lowering agents Percent yes; percent adding medication after baseline
- HIV-related health history (all based on site report at entry)
  - Time since HIV diagnosis (years): Median (Q1, Q3) (P10, P90)
  - Nadir CD4 count (cells/mm3): Percent by group (<50, 50-199, 200-349, ≥350, Unknown)
  - Total ART use duration (years): Percent by group (<5, 5-10, >10, Unknown)
  - Thymidine exposure: Percent yes
  - Abacavir exposure: Percent yes
  - TDF exposure: Percent yes
  - Protease inhibitor exposure: Percent yes

- HIV-related health at REPRIEVE entry
  - CD4 count (cells/mm3): Mean (SD)
  - HIV-1 RNA (copies/ml): Percent by group (<LLQ, LLQ-400, >400)
  - ART regimen: Percent by group (NRTI+INSTI, NRTI+NNRTI, NRTI+PI, NRTI-sparing, Other NRTI containing)
  - Entry NRTI: Percent by group (No NRTI, TDF, TAF, Abacavir, Other)
  - Entry INSTI: Percent by group (No INSTI, DTG, EVG, BIC, RAL)
- HIV-related health at Year 2
  - Most recent CD4 count (cells/mm3): Mean (SD)
  - Most recent HIV-1 RNA (copies/ml): Percent by group (<LLQ, LLQ-400, >400)
  - Change in class of ART from baseline: Percent yes
  - Current ART regimen: Percent by group (NRTI+INSTI, NRTI+NNRTI, NRTI+PI, NRTI-sparing, Other NRTI containing)
  - Change in NRTI from entry: Percent yes.
  - Current NRTI: Percent by group (TDF, TAF, Abacavir, No NRTI, Other)
  - Change in INSTI from entry: Percent yes.
  - Current INSTI: Percent by group (No INSTI, DTG, EVG, BIC, RAL)

7 Analysis Plan

7.1 General analysis Considerations

Outcome distributions will be described with median, Q1, Q3, P10, P90, minimum, and maximum and shown graphically. All primary treatment group comparisons will be performed ITT using a 5% type error. For continuous outcomes, inference will be based on differences in means adjusted for baseline and presented with two-sided, 95% confidence intervals.

7.2 Statin effects on coronary plaque morphology

Outcome measures: See Sections 3.2 and 3.3

Table: Primary and secondary plaque outcomes

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 linear regression with adjustment for baseline plaque volume and stratified by sex and CD4.

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, the probability of NCP and total plaque progression over two years (see Section 3.2.2) will be compared by treatment group using a stratified chi-squared test.

The statin effect on high risk plaque features (any), including low 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.

Sample table from Testosterone study, JAMA 2017

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Table: Modification of statin effect on changes in NCP and total plaque volume

- By baseline value, sex, age, screening CD4, and duration of suppressive ART

  Depending on their baseline prevalence, modification of the statin effect by use of cardiovascular medications may also be explored.

7.3 Statin effects on markers of immune function and markers of glucose homeostasis

Outcome measures: See Sections 3.4 and 3.5

Statin effects on the distributions of blood biomarkers belonging to distinct pathways will be assessed via treatment group comparisons of these respective markers via linear regression with adjustment for baseline and stratified by sex and CD4.

We will examine modification of statin effect on these markers by baseline value, sex, age, screening CD4, duration of suppressive ART, and presence of NCP at study entry.

Figure: Distributions of lipid fractions by treatment group over time

- Violin plots

Figure: Mean and 95% CI lipid fractions by treatment group over time

Table: Median, Q1, Q3 and Mean and 95% CI lipid fractions by treatment group over time

Sample table from Atorvastatin trial Lancet 2015

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7.4 Statin effects on lipids

Outcome measures: See Section 3.6.2

Figure: Distributions of lipid fractions by treatment group over time

- Violin plots

Figure: Mean and 95% CI lipid fractions by treatment group over time

Table: Mean and 95% CI lipid fractions by treatment group over time

7.5 LDL 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 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 upon adjustment for these biological factors.

Since the hypothesized mechanism is that sustained high levels of immune activation and inflammation precede and contribute to progression of NCP volume and high risk plaque 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.

Appendix I Sample Size Considerations

[Copied Verbatim from the REPRIEVE A5333S Protocol, Version 5.0, Section 9.2.]

The target sample size for the Mechanistic Substudy of REPRIEVE (A5333s) is 800 participants that will be 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 both with respect to plaque progression (among those with plaque at study entry) and rates of incident plaque (among those plaque-free at entry). Specifically, the total sample size of 800 subjects 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:

50% of study participants will have evidence of NCP at study entry [Lo 2010]
A SD of 20% for the percent change over 2 years among participants with evidence of plaque at entry
An annual rate of incident plaque development of 12% among participants without plaque at entry
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. Expectations for NCP prevalence and progression both as a whole and according to whether NCP was present at study entry are illustrated in the table below. These estimates are based on a simulation that further assumed the following:

Average volume NCP 250mm3 (SD=200) among participants with NCP at entry.
Average volume NCP 40mm3 (SD=20) at 2 years among participants without NCP at entry without statin treatment.
Average volume NCP 20mm3 (SD=20) at 2 years among participants without NCP at entry with statin treatment.
NCP distributions were also assumed to follow a gamma distribution with size and shape parameters determine to provide the desire mean and standard deviations.

Table 9.2-1: Cells constituting the primary targeted group comparisons are shown in bold.

		At study entry			At two 2 years						
		NCP (%)	NCP volume (mm3)		NCP (%)	NCP volume (mm3)		Change in NCP volume (mm3)		Percent change in NCP volume (%)[2]	
			Mean (SD)	[P5, P95]		Mean (SD)	[P5, P95]	Mean (SD)	[P5, P95]	Mean (SD)	[P5, P95]
Control	Overall[1]	50%	121 (186)	[0, 509]	59%	140 (210)	[0, 573]	19 (51)	[-21, 103]	17% (33%)	[-10%, 100%]
	No plaque at entry		-	-	**21%**	9 (19)	[0, 51]	9 (19)	[0, 51]	21% (41%)	[0%, 100%]
	No plaque at 2 years					-	-	-	-	-	-
	Plaque at 2 years					40 (20)	[17, 79]	40 (20)	[17, 79]	100% (0%)	[100%, 100%]
	Plaque at entry		251 (198)	[26, 637]		281 (231)	[28, 730]	30 (68)	[-38, 153]	**12% (20%)**	**[-14%, 49%]**
Statin	Overall[1]	50%	120 (185)	[0, 507]	52%	127 (198)	[0, 536]	7 (45)	[-42, 76]	7% (24%)	[-17%, 60%]
	No plaque at entry		-	-	**8%**	2 (8)	[0, 14]	2 (8)	[0, 14]	8% (27%)	[0%, 100%]
	No plaque at 2 years					-	-	-	-	-	-
	Plaque at 2 years					23 (19)	[2,60]	23 (19)	[2,60]	100% (0%)	[100%, 100%]
	Plaque at entry		250 (197)	[26, 633]		262 (216)	[26, 682]	12 (64)	[-66, 123]	**5% (20%)**	**[-21%, 43%]**

[1] *In estimation of overall means, participants without evidence of NCP are assigned a value of 0 for volume and change.*

[2] *In estimation of percentage change, participants without evidence of NCP at study entry with NCP at 2 years are assigned a value of 100%.*

These desired statin effects on NCP volume are similar to those seen in intravascular ultrasound (IVUS) studies [Nissen 2006; Nicholls 2005]. Although no data exist to directly inform the clinical relevance of these differences, randomized comparisons of high vs. low dose statin therapy among non-HIV-infected patients have demonstrated favorable effects on atherosclerotic plaque of a similar magnitude as well as on adverse cardiac events, among non-HIV patients [Nissen 2006]. Further, data from the "Coronary CT Angiography Evaluation for Clinical Outcomes (CONFIRM) registry suggest that the presence of non-obstructive CAD is predictive major adverse cardiac events independent of traditional risk factors, degree of stenosis and coronary artery calcification (HR 2-5) [Hulten 2013]. The proposed mechanistic study will further add to this body of evidence investigating, for the first time in HIV-infected population, the association of NCP (both the presence and magnitude) as well as changes in volume relate to events. For this exploratory analysis, a larger sample size will be beneficial, in order to accrue CVD events to relate to plaque morphology.

References

Hulten E, et al. Usefulness of coronary computed tomography angiography to predict mortality and myocardial infarction among Caucasian, African and East Asian ethnicities (from the CONFIRM [Coronary CT Angiography Evaluation for Clinical Outcomes: An International Multicenter] Registry). Am J Cardiol 2013;111(4):479-85. PMID: 23211358.

Lo J, et al. Increased epicardial adipose tissue volume in HIV-infected men and relationships to body composition and metabolic parameters. AIDS 2010;24(13):2127-30. PMID: 20588167.

Nicholls SJ, et al. Effect of atorvastatin (80 mg/day) versus pravastatin (40 mg/day) on arterial remodeling at coronary branch points (from the REVERSAL study). Am J Cardiol 2005;96(12):1636-9. PMID: 16360349.

Nissen SE, et al. ASTEROID Investigators. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. JAMA 2006;295(13):1556-65. PMID: 16533939.