This is PREPARE (ACTG A5361S) SAP Version 1.2 with names of authors redacted.

PREPARE (A5361S)

Pitavastatin to REduce Physical Function Impairment and Frailty in HIV (PREPARE)

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Protocol Version 2.0

PRIMARY STATISTICAL ANALYSIS PLAN

Version: 1.2

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Version History

Version	Changes Made	Date Finalized (mm/dd/yyyy)
1.0	Original Version	06/19/2017
1.1	Protocol amendment review (V1.0, LOA #3) - Title page updated according to the current CLIN.10070, and version history table added. - No changes to the content.	01/25/2021
1.2	Protocol amendment review (V2.0) - A COVID-19 related exploratory objective and a visit at Month 60 were added. While this increases the study duration from 48 months to 60 months (Section 4 - Protocol Overview), there are no changes to the monitoring activities.	08/05/2021

Table of Contents

1 INTRODUCTION

This is the primary statistical analysis plan (SAP) for PREPARE (A5361s), a sub-study of REPRIEVE (A5332). It describes the outcome measures and general analytic approaches proposed for the primary statistical analysis, and provides a forum for discussion and ultimate agreement between study team members. The outline of the report contents, tables and figures, specific coding details and data sources will be developed as a separate Analysis Implementation Plan, a supplement to SAP.

This analysis plan may be updated by the study team as new information becomes available outside of the study, and to modify the list of biomarker outcomes of interest closer to the time of analysis. In addition, the plans to evaluate mechanistic pathways through which pitavastatin affects physical function, including causal mediation analyses, will be developed closer to the time of analysis, given the expected developments in the relevant statistical methodology over the next four years.

Note that this is a team-monitored study. The monitoring reports on the study progress, conduct and data collection to the core study team members are outlined in the Study Monitoring Plan and are not part of this document.

2 GLOSSARY

AT	As-Treated
CM	Clarification Memo
DAG	Directed Acyclic Graph
DASI	Duke Activity Status Index
DMC	Data Management Center at Frontier Science (FSTRF)
HU	Hounsfield Unit
IIS	Inflammatory Index Score
ITT	Intention-To-Treat
LoA	Letter of Amendment
mSPPB	Modified Short Physical Performance Battery
REAP	Rapid Eating and Activity Assessment for Patients
SAP	Statistical Analysis Plan
SDAC	Statistical and Data Analysis Center
SPPB	Short Physical Performance Battery

3 DESIGN OVERVIEW

This section is based on the study protocol Version 1.0, clarification memo (CM) #1 and letter of amendment (LoA) #1.

Design Prospective, observational study of muscle strength and function among HIV-1 infected adults who are receiving pitavastatin or placebo as part of REPRIEVE (A5332). It is desired that a substantial proportion of study participants will also be enrolled in the Mechanistic Substudy of REPRIEVE (A5333s).

Location US clinical research sites (CRSs) that are participating in REPRIEVE (A5332) and its mechanistic substudy A5333s.

Brief Rationale With combination ART, longer life expectancy is changing the demographics of the HIV epidemic. However, even with long-term, effective ART, impairment in physical function and frailty are more common than expected and have been associated with an increased risk of falls, hospitalizations, and mortality. Furthermore, the combination of both HIV infection and impaired physical function is associated with a greater risk of mortality than the presence of HIV infection or impaired function alone; and systemic inflammation in HIV-infection has been shown to have a strong relationship with physical function impairment. Therefore, the aging HIV-infected population is an emerging risk group for the accelerated development of mobility disability, in urgent need for interventions to improve physical function and quality of life, possibly through interventions that attenuate the effects of inflammation and immune activation such as statins.

Sample size 600 participants.

The participants will be enrolled from the following pools:

1) Participants newly enrolling into REPRIEVE and A5333s (prospective enrollment from A5333s): Participants should enroll into REPRIEVE and PREPARE concurrently.
2) Participants already enrolled into REPRIEVE and A5333s (retrospective enrollment from A5333s): Participants can enroll into PREPARE within 24 months of their entry into REPRIEVE.
3) Participants newly enrolling into REPRIEVE who are not co-enrolling into A5333s (prospective enrollment from REPRIEVE): Participants should enroll into REPRIEVE and PREPARE concurrently.

Duration Up to 48 months, depending on the time of enrollment relative to REPRIEVE (A5332) entry (follow-up until 48 months from REPRIEVE entry).

Intervention None in A5361s. Pitavastatin or placebo for pitavastatin will be provided in REPRIEVE (A5332).

Primary Objective

(Numbered as in the study protocol.)

1.2.1 To determine whether pitavastatin can slow or prevent the decline in physical function of adults aging with HIV infection.

1.2.2 To evaluate mechanistic pathways through which pitavastatin affects physical function.

Secondary Objectives

(Numbered as in the study protocol.)

1.3.1 To characterize muscle function and muscle attenuation and their association with clinical characteristics and risk factors (such as age, comorbidities, level of physical activity, inflammation/immune activation, lipids) in virologically suppressed HIV-infected older (>45 years) population at low cardiovascular risk. *[Note that this secondary objective is intended for a baseline analysis after completion of enrollment and will be outlined in a separate plan prior to analysis of the baseline data.]*

1.3.2 To determine the effects of pitavastatin on muscle fat attenuation over 24 months.

1.3.3 To determine the relationship between changes in inflammation/immune activation and skeletal trunk muscle fat infiltration with change in physical function over 48 months.

1.3.4 To determine the impact of pitavastatin versus placebo on self-reported physical activity or sedentary time.

1.3.5 To assess the impact of muscle symptoms on the effect of pitavastatin versus placebo on physical function.

1.3.6 To determine whether changes in muscle fat attenuation are independent of lipid changes and changes in homeostasis model assessment-estimated insulin resistance (HOMA-IR).

Exploratory Objectives

(Numbered as in the study protocol.)

1.4.1 To determine whether the impact of pitavastatin on physical function or fat infiltration is affected by baseline vitamin D levels.

1.4.2 To determine whether the impact of pitavastatin on physical function or fat infiltration is affected by sex hormones (total and free testosterone) or menopausal status.

1.4.3 To determine the effects of pitavastatin on physical function by subgroup (race, sex, CD4 count, physical activity, baseline physical function, duration of antiretroviral therapy (ART), and inflammation).

4 OUTCOME MEASURES

For each outcome measure, the objectives which it will be used to address, are listed in brackets. Note that an outcome may be used in addressing multiple objectives.

4.1 Primary Outcome Measures

- Physical Function: Chair rise rate quantified annually over 48 months [1.2.1, 1.2.2, 1.3.3, 1.3.5, 1.4.1, 1.4.2, 1.4.3]. Participants are asked to perform 10 chair stands. Chair rise rate will be calculated as the number of chair stands performed divided by the time to perform chair stands. If participant is unable to complete 10 chair rises, 9 seconds will be used for each chair rise not completed (i.e. a total of 90 seconds for those who tried but were unable to complete any).
- Mechanistic Biomarker: Inflammatory Index Score (IIS) calculated as 1/3 log [IL-6] + 2/3 log [sTNFR-1] at REPRIEVE entry and 12 months. [1.2.2, 1.3.3]
- Mechanistic CT: Paraspinal muscle density (attenuation in Hounsfield units [HU]) at REPRIEVE entry and 24 months. [1.2.2, 1.3.2, 1.3.3, 1.3.6, 1.4.1, 1.4.2]

4.2 Secondary Outcome Measures

4.2.1 Physical Function and Activity Secondary Outcome Measures

All physical function outcome measures are assessed annually over 48 months (see Section 5), unless otherwise specified below.

Short Physical Performance Battery (SPPB) Outcome Measures [1.2.1]:

- Composite SPPB score (composite scoring of repeated chair stand, balance, and gait speed) *(Guralnik et al. J Gerontol. 1994 Mar; 49(2):M85-94.).* For gait speed, faster of the two results (minimum) will be used.
- Physical function deficits defined as a composite SPPB score <10.

Modified Short Physical Performance Battery (mSPPB) Outcome Measures [1.2.1]:

- Composite mSPPB score, modified slightly from Simonsick et al (J Gerontol A Biol Sci Med Sci (2001) 56 (10): M644-M649). The composite score (ranging from 0 to 3) defined as a sum of the following 3 components.
    - Chair rise rate (stands/second), as above, divided by maximal possible performance to derive a ratio between 0 to 1 (1 chair stand/second shown by previous studies will be used as the maximal possible performance unless suggested otherwise by the data).
    - Total standing balance time, as the sum of time to hold each of 3 stands (semi-tandem, tandem, and one-leg), divided by 90 (the allotted time of 30 seconds per each stand) to derive a ratio between 0 to 1.

  - Gait speed (meters/second) divided by the maximal possible performance to derive a ratio between 0 to 1 (2 meters/second shown by previous studies will be used as the maximal possible performance unless suggested otherwise by the data).
- Balance defined as ability to hold one leg stand for 30 seconds and as a ratio as defined above.
- Gait speed (meters/second) evaluated by time to complete a 4-meter walk. Average of the two results will be used.

Frailty Outcome Measures [1.2.1]:

Assessment of frailty is based on presence of the following criteria: (1) weight loss, (2) exhaustion, (3) low physical activity, (4) slow walk, (5) weak grip strength as described by Fried et al *(J Gerontol A Biol Sci Med Sci. 2001 Mar;56(3):M146-56.).* For gait speed and grip strength, average of the results will be used.

- Frailty classified as non-frail (no criteria present), pre-frail (1 – 2 criteria present), and frail (≥3 criteria present).
- Number of frailty components present.
- Presence of each frailty component (weight loss, exhaustion, low physical activity, slow walk, weak grip strength).
- Grip strength (kg) in the dominant hand (average of the results will be used).

Self-Reported Physical Activity and Capacity Outcome Measures [1.3.4]

- Self-reported physical activity via two questions measured on a three-point ordinal scale (usually/often, sometimes, rarely/never) on the Rapid Eating and Activity Assessment for Patients (REAP) assessment. In an average week, how often do you:
  - do less than 30 total minutes of physical activity 3 days a week or more? (Examples: walking briskly, gardening, golf, jogging, swimming, biking, dancing, etc.) [REAP question 26].
  - watch more than 2 hours of television or videos a day? [REAP question 27].
- Self-reported physical capacity measured by the overall score of Duke Activity Status Index (DASI) as described by Hlatky et al*. [Am J Cardiol. 1989 Sep 15;64(10):651-4.].* Evaluated at REPRIEVE entry and at month 24.

4.2.2 Mechanistic Biomarker Secondary Outcome Measures

Biomarker outcomes are quantified at REPRIEVE entry and at 12 months (see Section 5). They are used in addressing objectives 1.2.2 and 1.3.3 (Section 3).

- Serum concentration of biomarker IL-6.
- Serum concentration of biomarker sTNFR-1.
- Other biomarkers implicated in the pathogenesis of physical function impairment (these may include markers such as sTNFR-2, IP-10, IGF-1, TGF) or those that may mediate the effects of statin treatment on systemic inflammation (CMV IgG).

The specific list of biomarkers of interest may be modified closer to the time of analysis based on the identification of additional biomarkers or pathways involved in this process.

4.2.3 Mechanistic CT Secondary Outcome Measures

CT outcomes for fatty infiltration (muscle density) and muscle mass (area) are quantified at REPRIEVE entry and at 24 months (see Section 5). They are used in addressing objectives 1.2.2, 1.3.2, 1.3.3 and 1.3.6 (Section 3).

- Paraspinal muscle area.
- Trunk and shoulder muscle density (attenuation in HU).
- Trunk and shoulder muscle area.

5 DATA SOURCES FOR OUTCOME MEASURES

The data and samples from the main study REPRIEVE, the mechanistic substudy A5333s and PREPARE will be used as shown in the table below.

Outcome Measure	REPRIEVE (A5332) Entry	Year 1	Year 2	Year 3	Year 4
Physical function battery	A5361s[1]	A5361s[2]	A5361s	A5361s	A5361s
Frailty components	A5361s[1]	A5361s[2]	A5361s	A5361s	A5361s
Duke Activity Status Index (DASI)	A5332[3]		A5333s/A5361s[4]		
Self-reported physical activity and sedentary time (REAP)[5]	A5332[3]	A5361s[2]	A5333s/A5361s[4]	A5361s	A5361s
Myalgia symptom assessment	A5332[3]	A5332[3]	A5332	A5332	A5332
Samples for inflammatory markers	A5332[3]	A5332[3]			
Fasting lipids and insulin sensitivity	A5332/A5333s[3]		A5332/A5333s		
CT scan for fatty infiltration and muscle mass of the skeletal trunk muscles	A5333s[3]		A5333s		

[1] Will be available only to participants who enroll concurrently into REPRIEVE (A5332) and A5361s. For participants who enroll into PREPARE after REPRIEVE entry, evaluation will be conducted at PREPARE entry also.

[2] Will be available only to participants who enroll into A5361s concurrently or within 12 months of REPRIEVE (A5332) entry. For participants who enroll into PREPARE after REPRIEVE entry, evaluation will be conducted at PREPARE entry also.

[3] For participants who enroll into A5361s after REPRIEVE (A5332) entry, the data and samples collected in REPRIEVE and A5333s prior to A5361s entry will be used.

[4] For participants not enrolled into A5333s, collected as part of A5361s.

[5] Questions 26 and 27 only.

6 GENERAL ANALYSIS CONSIDERATIONS

6.1 Baseline

Baseline will be defined as the REPRIEVE entry evaluation. The baseline characteristics derived for the main study REPRIEVE will be used, if available. For the physical function and frailty measures, baseline is defined as PREPARE entry evaluation prior to or on the day of the study treatment initiation in REPRIEVE. As such, participants who enroll into PREPARE after initiation of study treatment in REPRIEVE, will not have a baseline (pre-treatment) evaluation.

6.2 Treatment Groups

Treatment groups (placebo vs. pitavastatin) will be defined according to randomization in REPRIEVE. Given the randomization and the double-blinding of treatment, there is no underlying difference expected between treatment groups among the PREPARE participants at baseline. Unless otherwise specified, all of the analyses outlined in this document will be performed by treatment group.

6.3 Time Variables

For derivation of time variables, month is defined as 30 calendar days (as in REPRIEVE) and year as 360 calendar days. Similarly, year will be defined as 360 calendar days in estimation of annual rate of change in an outcome measure.

6.4 Evaluations and Visit Windows

The schedule for visits and evaluations is shown in Section 5. PREPARE post-entry visits (months 12, 24, 36 and 48) are scheduled from REPRIEVE entry, preferably coinciding with the q12 month REPRIEVE visits but within 14 days of REPRIEVE visit is acceptable. The REPRIEVE study protocol specifies a window of ±21 days for these visits, with a re-scheduling within 7 days of the original visit if the participant is not fasting. For analyses of evaluations by study visits, broader windows of ±60 days will be used. If multiple evaluations are available within a window, the evaluation closest to the scheduled visit will be used. The study week will be calculated from the REPRIEVE entry date.

6.5 Analysis Population

All eligible randomized participants will be included in the analysis. Participants enrolled and later found ineligible will be evaluated on a case-by-case basis by the study team. Their inclusion or exclusion will be documented in the analysis report.

The analyses of treatment comparisons in the physical function outcome measures will be performed according to the principle of intention-to-treat (ITT), that is, participants will be analyzed according to their randomized treatment assignment regardless of subsequent changes to that treatment or the actual treatment received.

The primary treatment comparisons of biomarker and fatty infiltration outcomes will be as-treated (AT), that is, participants will be analyzed according to their actual treatment received and while on study treatment. Treatment interruptions will be examined and results will be excluded due to treatment interruptions for extended period of time, to be defined.

6.6 Missing Data

Given the strong plans for participant follow-up as part of the main study REPRIEVE, it is anticipated that missing data will be minimal. Missing data will be assumed to be missing at random. For participants lost to follow-up, the available outcome data until the time of loss to follow-up will be used. The amount of and reasons for missing evaluations of outcome measures and drop-out will be examined by treatment group. However, the longitudinal analysis of primary physical function outcome will be robust in the event of uneven participant drop-out related to the rate of decline in physical impairment.

6.7 Statistical Significance Level

Statistical comparisons of the primary outcome measures between treatment groups will be performed as two-sided at 5% Type I error.

Given the multiple primary hypotheses and the various secondary outcomes outlined, it is recognized that there is a multiplicity of analyses to be performed, which leads to an increased probability that at least one of the comparisons could be "significant" by chance. Although the overall level of significance for all treatment comparisons will be 5%, we will be conservative in the interpretation of the secondary and supporting analyses, taking into account the degree of significance, and looking for consistency across outcomes.

7 STUDY POPULATION

To describe the study population, the below baseline [REPRIEVE entry] information will be summarized.

The variables will be summarized as continuous, categorical or both, as indicated below. The tables will provide the number of participants, mean and standard deviation, median (Q1 – Q3), P10 – P90, minimum – maximum (referred to as statistics in the listings below) for continuous measures, and number (%) for categorical measures. In calculation of percentages, participants with missing data will be excluded from the denominator.

The baseline summaries will be provided by the following.

1) Overall and by treatment group.
2) By prospective vs. retrospective enrollment (enrolled into PREPARE before vs. after initiation of study treatment in REPRIEVE).
3) By co-enrollment into A5333s (i.e. participants with vs. without CT scans) and treatment group.

Summaries by treatment group will use randomized treatment group.

For categorical variables, Chi-squared test (all cells have at least 5 expected counts) or Fisher's Exact Test (at least one cell has less than 5 expected counts) will be used to compare prospective vs. retrospective enrollees (#2 above) and those enrolled vs. not enrolled into A5333s (#3 above). Wilcoxon rank sum tests will be used for continuous variables. Since treatment allocation was randomized and double-blinded in REPRIEVE, no statistical tests will be provided for the summaries by treatment group.

Demographic and behavioral variables:

Variable	Summary	Details
Age (years)	Statistics	Age at REPRIEVE entry.
	Number (%)	By category: 40-49, 50-59, 60-69, 70-75 years.
Sex	Number (%)	Defined as sex at birth.
Race	Number (%)	
Ethnicity	Number (%)	
Race/ethnicity	Number (%)	
Menopausal status	Number (%)	Limited to women.
Weight (kg)	Statistics	
BMI (kg/m^2)	Statistics	Calculated as weight (kg)/height(m)^2.
	Number (%)	By category: as underweight (<18.5), normal (18.5-24.9), overweight (25-29.9), obese (≥30).
Smoking status	Number (%)	By category: current; former; never.
Total years smoked	Statistics	Limited to former/current smokers.
Alcohol use	Number (%)	By category: yes/no, defined as: yes = sometimes or often drink 1-2 alcoholic drinks a day; no = rarely/never.

HIV-related variables and ART regimen:

Variable	Summary	Details
Nadir CD4 (cells/mm^3)	Statistics	By category: <50; 50-99; 100-199; 200-349; 350-499; ≥500; Unknown.
CD4 (cells/mm^3)	Statistics	
	Number (%)	By category: 50-199, 200-349, 350-499, 500-649, 650-799, ≥800.
HIV-1 RNA (copies/ml)	Number (%)	By category: <50; ≥50.
	Statistics	Limited to those with quantifiable HIV-1 RNA, if substantial number
ART regimen	Number (%)	By class (NRTI+NNRTI, NRTI+PI, NRTI+INI, Other NRTI-containing, NRTI-sparing) and regimen.
ART duration (years)	Statistics	
	Number (%)	By category: <10; ≥10.

Co-morbid conditions:

Variable	Summary	Details
Years since HIV diagnosis	Statistics	
History of AIDS	Number (%)	
HBV/HCV	Number (%)	
Hypertension	Number (%)	
Diabetes mellitus	Number (%)	
Cancer	Number (%)	
Chronic kidney disease	Number (%)	
Family history of CVD	Number (%)	

8 EFFECT OF PITAVASTATIN ON PHYSICAL FUNCTION

This section outlines analyses for the outcomes addressing the primary objective 1.2.1, secondary objectives 1.3.4, 1.3.5 and exploratory objective 1.4.3 (see Section 3).

8.1 Analysis Considerations

The analyses of physical function outcomes will be ITT, as defined in Section 6.5.

Descriptive statistics (number of observations, mean and standard deviation, median (Q1 – Q3), P10 – P90, minimum – maximum) for numerical outcomes, number (%) for categorical outcomes and ordinal outcomes with limited scale (e.g. frailty with 3 ordered categories), and graphical presentations will be examined at REPRIEVE entry and over time. PREPARE entry evaluations performed after the start of REPRIEVE study treatment will not be used for REPRIEVE entry in these summaries.

8.2 Primary Analysis of the Chair Rise Rate

8.2.1 Analysis Data

All available outcome data regardless of participants time of entry into PREPARE (i.e. including both, PREPARE entry evaluations performed prior to and after REPRIEVE study treatment initiation) and regardless of visit windows. Participants without pre-treatment chair rise rate will be included.

8.2.2 Analytic Methods

Linear mixed effects model will be used, with time modeled on a continuous scale, where REPRIEVE entry is time 0. The primary model will include intercept, the main effect of time and the interaction of time and treatment group, and omit the main effect of treatment group (intercept). It takes advantage of the fact that randomized group allocation in REPRIEVE means that there is no underlying difference in physical function between treatment groups at REPRIEVE entry and will be the most powerful *[Fitzmaurice GM, Laird NM, Ware JH. Applied Longitudinal Analysis. 2nd ed. Hoboken, New Jersey: John Wiley & Sons, Inc, 2011].* Exchangeable correlation structure will be used for repeated measurements within participant. The primary parameter of interest is the difference in the rate of change (slope) between placebo and pitavastatin over time (i.e., the interaction between treatment and time).

In the event of non-linear change in outcome over time, alternative transformations of the outcomes or time axis will be assessed. If such transformations are not feasible, modeling will be performed using an arbitrary time effect (i.e., time modeled as a factor on four levels). The treatment effect of interest for the primary treatment group comparison will be the average difference across post-baseline time points; an interaction of treatment and time will also be assessed and secondary comparisons by year will also presented.

8.3 Subgroup Analyses of Chair Rise Rate

These primary analyses will be extended to evaluate subgroup effects of treatment, namely different treatment effects by

- race (white vs non-white),
- sex,
- baseline CD4 cell count (<350 cell/mm^3 vs. ≥350 cells/mm^3),
- ART duration (<10 or ≥10 years),
- baseline physical function deficits (mSPPB <10 vs. ≥10),
- baseline systemic inflammation (by tertile of IIS).

The basic model will include intercept, the main effects of time and subgroup and the interaction of time and subgroup, i.e. incorporating potential differences between subgroups both, in baseline chair rise rate, and in rate of change per year. To evaluate subgroup effects of treatment, inclusion of the interaction of

time and treatment group, and the interaction of time, treatment group and subgroup will be considered. The primary parameter of interest is the difference in the rate of change (slope) between placebo and pitavastatin over time between the levels of a subgroup variable (i.e., the interaction between treatment, time and subgroup). Each subgroup analysis will be conducted separately. The model will not be extended to include multiple subgroup variables.

8.4 Sensitivity Analyses of Chair Rise Rate

As a sensitivity analysis, the primary analysis will be conducted excluding participants who enrolled into PREPARE after REPRIEVE entry (i.e. participants who enrolled into PREPARE retrospectively and do not have pre-treatment chair rise rate available). These sensitivity analyses will not be conducted for the subgroup analyses, but they may be considered should there be a substantial difference found in the results of the primary treatment effect analysis.

8.5 Analyses of Secondary Physical Function Outcomes

This section describes analyses of the secondary physical function and activity outcomes listed in Section 4.2.

8.5.1 Analysis Data

Available outcome data with the exception of PREPARE entry evaluations performed after initiation of study treatment not used for baseline. The evaluations will be separated into baseline, and months 12, 24, 36 and 48 as specified in Section 6.4. In the linear mixed effects model and cumulative logit model, all evaluations will be used regardless of participants time of entry into PREPARE and visit windows.

8.5.2 Analytic Methods

For the secondary outcomes (including gait speed, grip strength, composite mSPPB ratio and balance ratio), linear mixed effects modeling approach as described for the primary analysis (Section 8.2.2) will be used, if appropriate, given the data distributions. This will provide estimates of the difference in the rate of change (slope) in gait speed (meters/second per year) and grip strength (kg per year) between placebo and pitavastatin over time. For some outcome measures (e.g. composite mSPPB ratio and balance ratio), log-transformation may be used to provide estimates of annual change in percent scale.

Treatment group differences in the prevalence of the frailty phenotype (non-frail, pre-frail, frail) will be assessed via marginal modeling with a cumulative logit using generalized estimating equations. Proportional odds approach which extends inference to underlying continuum and is invariant with respect to choice of response categories will be used.

Similarly to the primary analysis of linear mixed effects model (Section 8.2.2), time will be modeled on a continuous scale, where REPRIEVE entry is time 0 and the model will include intercept, the main effect of time and the interaction of time and treatment group, and omit the main effect of treatment group (intercept). Exchangeable correlation structure will be used for repeated measurements within participant. The main parameter of interest is the difference in the rate of change (slope) between placebo and pitavastatin over time (i.e., the interaction between treatment and time).

Given the discretized distribution of the remaining physical function secondary outcomes (including the composite SPPB score and overall DASI score), Wei and Johnson method will be used to assess the overall difference between treatment groups over time.

9 EFFECT OF PITAVASTATIN ON INFLAMMATION/IMMUNE ACTIVATION AND SKELETAL TRUNK MUSCLE

This section outlines analyses for the outcomes addressing the secondary objectives 1.3.2 and 1.3.6, and for the effect of pitavastatin on mechanistic biomarker outcomes which are not described in A5333s. Refer to Section 4 for the list of outcome measures.

9.1 Analysis Considerations

The analyses of mechanistic biomarker and fatty infiltration outcomes will be AT, as defined in Section 6.5.

Descriptive statistics (number of observations, mean and standard deviation, median (Q1 – Q3), P10 – P90, minimum – maximum) and graphical presentations over time will be provided.

9.2 Analysis Data

Outcome measure results at baseline and post-baseline on-treatment results. Results after treatment interruptions for extended period of time (to be defined) will also be excluded (Section 6.5). The evaluations will be separated into baseline, and month 12 for biomarkers or month 24 for CT outcomes (fatty infiltration and muscle mass) as specified in Section 6.4. The analyses of the CT outcomes include (1) all A5333s participants with results available (i.e. including those not enrolled into PREPARE) (2) participants co-enrolled into PREPARE and A5333s with results available.

9.3 Analytic Methods

Pointwise treatment group comparisons will use linear regression adjusted for baseline to provide estimation of the difference in the baseline-adjusted level of the outcomes at the follow-up visit. Outcome transformations will be applied as appropriate. Treatment group comparisons of markers of inflammation and immune activation will be limited to those not described in A5333s. For the CT outcomes, the primary treatment comparisons are on all participants with the muscle function data (i.e. including those not enrolled into PREPARE). The analysis will also be conducted among the participants enrolled into PREPARE to ensure a consistent effect size. Unless a test for heterogeneity of effect (interaction between enrollment into PREPARE and treatment effect) suggests otherwise, the PREPARE cohort effect sizes will be presented to give content to the main findings, but inference will be made on the full cohort.

Analyses of CT outcomes will also assess the association of these changes with changes in lipid components and HOMA-IR as listed below. The linear regression for treatment group comparison described above will be expanded to evaluate each lipid component and HOMA-IR separately.

- Lipid components including LDL cholesterol and triglycerides
- HOMA-IR calculated as [glucose (mg/dL) x insulin (uIU/mL)]/ 405

10 MECHANISTIC PATHWAYS THROUGH WHICH PITAVASTATIN AFFECTS PHYSICAL FUNCTION

This section outlines analyses for the outcomes addressing the primary objective 1.2.2, secondary objectives 1.3.3, 1.3.5, and exploratory objectives 1.4.1 and 1.4.2. The primary physical function outcome measure of chair rise rate and all mechanistic biomarker and fatty infiltration outcome measures will be used (Section 4).

10.1 Analysis Considerations

The analyses will be ITT, as defined in Section 6.5.

10.2 Analysis Data

The results for the primary physical function outcome, chair rise rate, will be used. Biomarker and CT outcome results from baseline and post-baseline (month 12 or month 24 for biomarkers and CT, respectively). The analyses using biomarker outcomes from A5333s and CT outcomes are limited to participants who were co-enrolled in A5333s.

10.3 Analytic Methods

The relationship between markers of inflammation/immune activation, fatty infiltration, and physical function will be assessed via longitudinal modeling of the chair rise rate (dependent variable) and expanding each of the appropriate models used to assess treatment group difference including baseline and post-baseline biomarker and fatty infiltration outcomes. Directed acyclic graphs (DAGs) will be used to describe the assumed causal relations among the biomarkers, fatty infiltration outcomes, other potential covariates (see below), and chair rise rate to guide in covariate selection.

The analyses will also examine associations with the following measured covariates/risk factors:

- Demographic and behavioral variables (age, race, sex, BMI, smoking, alcohol use, physical activity by REAP)
- Co-morbid conditions (HBV/HCV, hypertension, diabetes mellitus, chronic kidney disease)
- Menopausal status and sex hormones (total and free testosterone), for women only; depending on the number of women enrolled
- Baseline vitamin D level
- Use of angiotensin-converting-enzyme inhibitor/angiotensin II receptor blocker (ACE-I/ARB) therapy
- HIV-related variables (CD4 cell count [nadir and current]), HIV RNA, antiretroviral exposure (cumulative and current exposure to ART)
- Muscle symptoms (via the myalgia symptom assessment collected through REPRIEVE)

- Concomitant medications, specifically, ACE-I/ARB therapy and use of hormonal therapy

Given the assumed temporal associations under study, the analyses will consider only including dependent outcomes assessed from month 24 onward. These analyses will be considered with and without adjustment for treatment assignment to allow assessment of the degree to which the effects of treatment might be mediated through treatment-associated changes. While further, more formal causal mediation analyses are planned, given the expected statistical developments in the relevant statistical methodology over the next 4 years, these analysis plans will be developed closer to the time of analysis.