A5350 Statistical Analysis Plan

Safety, Tolerability, and Effects of the Probiotic Visbiome Extra Strength on Gut Microbiome and Immune Activation Markers in HIV-Infected Participants on Suppressive Antiretroviral Therapy

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This is ACTG A5350 SAP Version 2.0 with names of authors, names of publication writing team members and analysis timeline redacted

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1 Introduction

This document presents the proposed primary, secondary, and exploratory statistical analyses of ACTG A5350. It also presents analyses which will be included in reports for the Study Monitoring Committee (SMC) interim review(s). This analysis plan may be modified by the study team as necessary; major modifications will be clearly indicated.

2 Study Overview

2.1 Design

A5350 is a phase II, randomized, double-blind, two-arm study to evaluate whether there is a significant change in sCD14 after 24 weeks of probiotic Visbiome Extra Strength therapy, and to determine the safety and tolerability of this agent in HIV-infected participants on stable antiretroviral therapy (ART). Participants will be followed for an additional 12 weeks off study product.

A substudy, A5352s will be conducted in a subset of participants to collect colonic biopsies via flexible sigmoidoscopy, and to evaluate intestinal permeability and inflammation at two time points.

2.2 Duration

Thirty-eight (38) weeks for each participant.

2.3 Sample Size

Ninety (90) participants (45 in Arm A and 45 in Arm B)

2.4 Population

HIV-infected participants (≥18 years of age) who have been on stable ART for at least 24 weeks prior to study entry, and have a CD4+ T-cell count >200 cells/mm^3 prior to study entry, and plasma HIV-1 RNA <50 copies/mL for 48 weeks prior to study entry.

2.5 Stratification

By intent and eligibility to enroll in the substudy A5352s.

2.6 Regimen

Participants will be randomized in a 1:1 ratio to either Arm A (Visbiome Extra Strength + stable ART; treatment arm) or Arm B (placebo for Visbiome Extra Strength + stable ART; control arm).

Arm A: Visbiome Extra Strength
2-week lead-in period: 1 Visbiome Extra Strength sachet per day.
Then increase to 1 sachet twice daily for 22 weeks.
Followed by 12 weeks of additional follow-up.

Arm B: Placebo for Visbiome Extra Strength
2-week lead-in period: 1 placebo sachet per day.
Then increase to 1 sachet twice daily for 22 weeks.
Followed by 12 weeks of additional follow-up.

2.7 Primary Objective

I. To assess changes in sCD14 in participants treated with Visbiome Extra Strength compared to placebo.

2.7.1 Hypothesis

I. After administration of Visbiome Extra Strength, systemic levels of sCD14 will decrease.

2.8 Secondary Objectives

I. To assess changes in additional markers of systemic inflammation (IL-6, IP-10, sCD163, sTNF-RI, oxidized LDL), KT ratio, and coagulation (D-dimer) after Visbiome Extra Strength administration.

II. To assess changes in markers of microbial translocation (LPS, LBP) after Visbiome Extra Strength administration.

III. To assess changes in peripheral CD4+ lymphocyte counts and CD4+/CD8+ ratio after Visbiome Extra Strength administration.

IV. To assess markers of monocyte and lymphocyte activation and senescence after Visbiome Extra Strength administration.

V. To assess change in gastrointestinal microbial diversity from fecal samples after Visbiome Extra Strength administration.

VI. To assess measures of enterocyte death (I-FABP) after administration of Visbiome Extra Strength.

VII. To assess safety and tolerability of Visbiome Extra Strength.

VIII. To assess diversity of the gut microbiome 12 weeks after completion of 24 weeks of Visbiome Extra Strength.

2.8.1 Hypotheses

I. After administration of Visbiome Extra Strength, other markers of systemic inflammation interleukin (IL)-6, interferon gamma-induced protein (IP)-10, soluble tumor necrosis factor receptor I (sTNF-RI), soluble CD163 (sCD163), oxidized low-density lipoprotein (LDL), kynurenine to tryptophan (KT) ratio, and coagulopathy (D-dimer) will decrease.

II. After administration of Visbiome Extra Strength, markers of microbial translocation lipopolysaccharide (LPS) and lipopolysaccharide-binding protein (LBP) will decrease.

III. After administration of Visbiome Extra Strength, peripheral CD4+ lymphocyte counts and CD4+/CD8+ ratio will increase.

IV. After administration of Visbiome Extra Strength, markers of lymphocyte and monocyte activation and senescence will decrease.

V. Visbiome Extra Strength administration will increase gastrointestinal microbial diversity in fecal samples.

VI. After administration of Visbiome Extra Strength, markers of enterocyte death as measured by I-FABP (intestinal fatty acid binding protein) will decrease.

VII. Visbiome Extra Strength will be safe and well tolerated.

VIII. The gut microbiome changes will persist after completion of Visbiome Extra Strength administration.

2.9 Exploratory Objectives

I. To assess changes in a marker of insulin sensitivity (HOMA-IR) after administration of Visbiome Extra Strength.

II. To assess changes in fasting lipid parameters (low-density lipoprotein [LDL], high-density lipoprotein [HDL], non-HDL cholesterol, and triglycerides) after administration of Visbiome Extra Strength.

III. To explore the correlations between the gut microbiome and soluble and cellular markers performed in the study.

IV. To explore the correlations between the microbial translocation markers and other soluble and cellular markers performed in the study.

V. To assess durability of the probiotic effect on inflammatory and microbial translocation markers 12 weeks after discontinuation of Visbiome Extra Strength therapy.

VI. To assess the differences in composition of the baseline microbiome based on ART.

VII. To assess changes in chronic gastrointestinal symptom score after administration of Visbiome Extra Strength.

VIII. To assess the relationship between reported dietary intake and composition of the microbiome.

2.9.1 Hypotheses

I. After administration of Visbiome Extra Strength, insulin sensitivity, as measured by homeostatic model assessment (HOMA-IR), will improve.

II. After administration of Visbiome Extra Strength, fasting lipid parameters will improve.

III. After administration of Visbiome Extra Strength, changes in gut microbiome will be associated with changes in markers of systemic inflammation and coagulation as well as changes in cellular immune activation markers.

IV. After administration of Visbiome Extra Strength, changes in markers of microbial translocation will be associated with changes in systemic markers of inflammation and coagulation as well as changes in cellular immune activation markers.

V. The improvements in markers of systemic inflammation and microbial translocation will persist for 12 weeks after completion of 24 weeks of Visbiome Extra Strength.

VI. At baseline, the composition of the microbiome will differ by antiretroviral class including protease inhibitors (PIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and integrase inhibitors (InSTIs).

VII. After administration of Visbiome Extra Strength, gastrointestinal symptom scores will improve.

VIII. The composition of the microbiome will be influenced by dietary intake as reported through the dietary assessments.

2.10 Protocol History

I. Protocol Version 1.0 (finalized January 5, 2016)

II. Protocol Version 1.0 Clarification Memo #1 (February 22, 2016) clarifying that all A5350 on-treatment visits must be fasting, adding to items that were inadvertently left out of the protocol, and confirming that fasting is required for the A5352S lactulose/mannitol visits.

III. Protocol Version 1.0 Letter of Amendment #1 (April 15, 2016) specifying the pregnancy test sensitivity and defining reproductive potential in A5350 and removed Week 24 hematology evaluations in A5352S.

2.11 Monitoring

2.11.1 Team Monitoring

Accrual, a summary of AEs, and sample/data availability will be reviewed regularly by the protocol core team once initial programming is complete. This summary will be pooled over the study arms. In addition, baseline characteristics as well as premature study treatment and study discontinuations (and reasons) will be reviewed regularly by the protocol core team.

2.11.2 Planned SMC Reviews

Per the protocol, an ACTG-appointed Study Monitoring Committee (SMC) will review accrual, adverse event summaries, off-treatment and off-study rates and reasons, and sample/data availability (if available from the DMC), broken down by study arm. The first SMC review will occur 6 months after the first participant is enrolled and then every 6 months as long as participants remain in follow-up. In addition, longitudinal changes in CD4+ T-cell count and HIV-1 RNA levels will be reviewed. Note that stored specimens will be assayed in batches after follow-up is concluded, and, therefore these data will not to be available at interim reviews. The SMC may also be convened if a reason is identified by the DAIDS clinical representative, study chairs, or study statistician in consultation with the team.

Open reports (pooled over treatment arms) will be distributed to the SMC and team members participating in the SMC review. In addition a closed report (by treatment arm) will be distributed to the SMC and study statisticians. The following summarizes the components of these reports:

Open Report:

- Screening and Entry Analyses
- Study Status and Extent of Follow-up Analyses
- Study Treatment Status
- Data Completeness (if available from the DMC)
- Immunologic and Virologic Studies
- Safety

Closed Report:

- Screening and Entry Analyses
- Study Status and Extent of Follow-up Analyses
- Study Treatment Status
- Data Completeness (if available from the DMC)
- Immunologic and Virologic Studies
- Safety

2.11.3 SMC Review History

From SMC Review Letter:

"The SMC met via teleconference on Wednesday, October 26, 2016, to review the interim safety reports. After carefully reviewing the accrual and safety data for both A5350 and A5352s, the SMC concluded that no significant safety concerns were identified and the studies should proceed as planned."

3 Outcome Measures

3.1 Primary Outcome Measure

I. Change in sCD14 from baseline to week 26.

3.2 Secondary Outcome Measures

I. Change in the following markers from baseline to week 26.
   a. Systemic inflammation markers (IL-6, IP10, sCD163, TNF-RI, oxidized LDL), kynurenine to tryptophan (KT) ratio, and coagulation marker (D-dimer).
   b. Microbial translocation markers (LPS, LBP).
   c. CD4+ lymphocyte counts and CD4+/CD8+ ratio.
   d. Monocyte (%CD14+CD16-, %CD14+CD16+, %$CD14^{low}CD16^{hi}$) and lymphocyte (%CD38+, %HLA-DR+, %CD38+HLA-DR+ in CD4+ and CD8+) activation and senescence (%CD28-CD57+ in CD4+ and CD8+).
   e. Gastrointestinal microbial diversity (Shannon index, richness) from fecal samples. [***Not analyzed at SDAC***]
   f. Enterocyte death (I-FABP).

II. Occurrence of adverse events.

III. Diversity of gut microbiome (Shannon index, richness) at week 38. [***Not analyzed at SDAC***]

3.3 Exploratory Outcome Measures

I. Change from baseline to week 26 in HOMA-IR.

II. Change from baseline to week 26 in LDL, HDL, non-HDL cholesterol, and triglycerides.

III. Change from week 26 to week 38 in systemic inflammation markers (IL-6, IP10, sCD163, sTNF-RI, oxidized LDL, and KT ratio).

IV. Gastrointestinal microbial diversity from fecal samples at entry and post-entry visits. [***Not analyzed at SDAC***]

V. Change in gastrointestinal symptoms from baseline to 26 weeks.

VI. Diversity of gut microbiome and dietary intake composition. [***Not analyzed at SDAC***]

4 Statistical Methods

4.1 Analysis Populations

Intent-To-Treat (ITT): All randomized participants.

Modified Intent-To-Treat (mITT): All randomized participants who started study product.

Per-Protocol (PP): All eligible, randomized participants who 1) have baseline and week 25/26 sCD14 measurements, 2) remain on study product through week 26 (participants with more than 50% on average of missed study product may be excluded), 3) have not used prohibited medications, 4) do not have a confirmed virologic failure (as defined per section 6.2.3 at or prior to week 26, and 5) do not experience inflammatory conditions, receive vaccines, or have concurrent illness (as defined per section 6.2.3).

4.2 Participant Exclusion

Participants found to be ineligible for A5350 by the site, study statistician(s), or data manager will be reviewed by the study co-chairs for confirmation of their ineligibility.

4.3 Analysis Visit Windows

For all analyses, the following visit windows will be used:

- **Screening**: evaluations completed within 45 days of randomization.
- **Entry (week 0)**: entry evaluations must occur on/after randomization and at least 24 hours after screening.
- **Week 2**: pre-treatment evaluation scheduled within a week [1, 3] window but prior to treatment initiation, treatment must begin within the next 72 hours after evaluation.
- On-treatment evaluations:
    - **Week 4:** weeks (3, 5]
    - **Week 6:** weeks (5, 8]
    - **Week 14:** weeks [12, 16]
    - **Week 25 & 26:** weeks [23, 28]
- **Week 38**: post-treatment evaluation scheduled within a week [36, 40] window.

If multiple results are available within the Week 6 or 14 visit windows the result closest to the scheduled visit will be used. If multiple results are available within the Week 38 window the result closest to 12 weeks after the last dose of study treatment (corresponding to protocol-defined 12 week washout) will be used.

Results within the weeks [23, 28] visit window will use the following logic to determine Week 25 and Week 26 results:

- If two results are collected on treatment the earlier will be Week 25 and the latter Week 26.
- If two results are collected off treatment the earlier will be Week 25 and the latter Week 26.
- If one result is collected on treatment and one result is collected off treatment the on treatment will be Week 25 and the off treatment Week 26.
- If only one result is collected it will be Week 25.
- If three or more results are available priority will be given to those collected on treatment and assigned Weeks based on the above rules.

For markers run in duplicate at entry and Week 2 and also at Weeks 25 and 26, “baseline” will refer to the average of entry and Week 2 and “Week 25/26” will refer to the average of Week 25 and Week 26. If only one result is available at either of these time points the single result will be used. For per-protocol analyses the “Week 25/26” calculation will only use results collected while on treatment.

4.4 Statistical Summaries and Testing

All statistical tests will be two-sided with a nominal alpha level of 0.05 and no adjustment for multiple testing.

Specimens collected 1 day after the last dose of study treatment will be considered “on treatment”.

Specimens collected within 6 days of an influenza vaccination will not be used for analysis unless the vaccination and specimen collection occurred on the same date and the site has documented that the vaccination was given after specimen collection.

Outcome measures will be transformed for analyses and summaries on the $\log_{10}$ scale, as appropriate, if determined to not be approximately normally distributed.

Because the stratification is to ensure balance between the treatment arms within A5352s and is not felt to affect A5350 outcomes, the stratification will not be taken into account in analyses.

Safety analyses will summarize the highest grade AE by participant using the mITT population and will compare treatment arms using the Wilcoxon rank-sum test. All AEs and AEs related to study treatment will be analyzed.

For all continuous outcome measures, tabular and graphical data summaries will be provided for both cross-sectional values across study weeks and change from baseline to post-baseline study weeks and will use the PP population. Mean changes in outcome measures will be compared between arms with either a two-sample t-test with equal variances or an adjusted linear regression model, as appropriate. The mean difference and 95% confidence interval (CI) will be provided along with the associated p-value.

For the primary analysis, two supplemental linear regression analyses will be performed. The first will additionally adjust for baseline sCD14 values (continuous) while the second will assess differential Visbiome Extra Strength

effects by baseline sCD14 tertile by additionally adjusting for the sCD14 tertile main effect and the study arm by sCD14 tertile interaction.

Correlations will be assessed with scatter plots as well as the Spearman correlation coefficient and associated p-value using the PP population.