ACTG A5337

Safety and Efficacy of Sirolimus for HIV Reservoir Reduction in Individuals on Suppressive Antiretroviral Therapy

ClinicalTrials.gov Identifier: NCT02440789

Statistical Analysis Plan

Version 5.0

May 9, 2019

This is ACTG A5337 SAP Version 5.0 with names of authors and names of publication writing team members redacted.

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1 INTRODUCTION

This document describes the content proposed for the primary statistical analysis of ACTG A5337. The focus is on analyses that address the key safety, tolerability and efficacy outcome measures, including those needed to address the study's primary and major secondary objectives. This analysis plan forms the core of any presentation or publication used to disseminate the primary conclusions of the study. It is, however, recognized that this analysis plan may be modified**/expanded** by the study team as new information becomes available outside of the study, or **based on the observed findings of this study**.

This analysis plan is on the study's primary and major secondary objectives, as well as the exploratory objective on soluble markers. Analyses of exploratory objectives not addressed in this document will start when those data become available. Results of the primary and secondary objectives addressed in this document will be submitted to clinicaltrials.gov within one year of the primary completion date, with the exception that laboratory assays for some of these outcomes might not be available in this timeframe and hence, an extension would be requested to clinicaltrials.gov.

2 UPDATES AND MAJOR CHANGES FROM VERSION 1.0

Version 2.0 contains the following updates

- **Removed details of exploratory objectives other than soluble biomarkers**
- **Removed details of interim monitoring, SMC reviews, and details of program validation and double coding**
- **Additional details are provided for secondary objectives because the laboratory testing plans and time points are now finalized**

Version 3.0 contains the following updates

- **T-Cell activation, proliferation and subset markers have been updated to reflect the flow panel implemented by the testing lab**
- **Based on discussions with the study immunologist, the derived flow-based outcomes have been separated into a set of secondary outcomes and a larger set of exploratory outcomes**
- **Added details regarding analyses for the primary and secondary efficacy populations**

Version 4.0 contains the following updates

- **Addition of other (exploratory) outcomes: CD8+ T-Cell counts and CA-RNA/CA-DNA Ratio**
- **Updated approach to assess differences in baseline time points for primary outcomes**

Version 5.0 contains the following updates

- **Added/updated secondary and other stimulated flow outcomes**
- **Added details of PK analysis**

Note: Bolded text is used throughout this document to indicate major changes from version 1.0.

Version	Changes Made	Date finalized
5.0	- Added signature page and version history table - Updated and added details of stimulated flow outcomes and PK analysis	5/9/2019

3 STUDY SCHEMA, HYPOTHESIS AND OBJECTIVES

Note: The following material is extracted from the study protocol Version **2.0, L.O.A. #1 and C.M. #1 and 2**.

STUDY SCHEMA

DESIGN	A5337 is a phase I/II, open label, single arm, pilot study to evaluate the safety of sirolimus and its efficacy with respect to its effects on HIV-1 reservoir size and immune function. This study will assess the effects of sirolimus on HIV-1-specific CD8+ T-cell function, HIV transcription, and residual viral production. Measurement of additional inflammatory markers, immunological studies, and reservoir size will be performed on stored specimens if the initial findings appear promising.
DURATION	44 weeks (12-week pre-sirolimus treatment lead-in period, followed by 20 weeks of sirolimus treatment and an additional 12 weeks off sirolimus treatment).
SAMPLE SIZE	30 participants **Target enrollment was 30 participants in order to achieve n = 25 evaluable participants for the as-treated primary efficacy analysis population.**
POPULATION	HIV-infected men and women ≥18 years of age, maintained on suppressive antiretroviral therapy (ART) for ≥24 months with CD4+ cell count ≥**350** cells/mm^3. **Participants** may not be on a PI-based or cobicistat-based regimen 3 months prior to and at any time after study entry and must remain on ART during sirolimus study treatment.
STRATIFICATION	**By class of antiretroviral (ARV) regimen**
REGIMEN	For **participants** on a non-protease inhibitor (PI), non-non-nucleoside reverse transcriptase inhibitor (NNRTI) regimen, and for those on a non-PI, rilpivirine (RPV) based regimen: Sirolimus 0.025 mg/kg/day initial dose for 20 weeks For **participants** who are on an NNRTI regimen with the exception of RPV: Sirolimus 0.05 mg/kg/day initial dose for 20 weeks. Dosing will be adjusted based on trough sirolimus concentrations to achieve target concentrations between 5 and 10 ng/mL.
HYPOTHESES	Sirolimus will be safe and tolerated in **participants** on non-protease inhibitor (PI) suppressive antiretroviral therapy (ART). Sirolimus will improve HIV-specific cellular immunity and reduce T-cell activation, which in turn will cause decreases in viral transcription/cell-associated RNA levels and levels of low-level residual viremia. Sirolimus will cause down-regulation of programmed cell death protein (PD-1) and suppression of homeostatic proliferation of the latent reservoir, leading to reductions in cell-associated HIV-1 DNA levels.

PRIMARY OBJECTIVES (NUMBERED AS IN PROTOCOL)

1.2.1 To assess the safety of sirolimus in HIV-infected participants receiving non PI-based suppressive ART.

1.2.2 To evaluate changes in the frequency (%) of HIV-1 Gag-specific CD8+ cells after 20 weeks of sirolimus therapy.

1.2.3 To assess changes in CD4+ cell-associated HIV-1 RNA and plasma HIV-1 RNA by single-copy assay (SCA) at baseline and at week 20 of sirolimus therapy.

SECONDARY OBJECTIVES (NUMBERED AS IN PROTOCOL)

1.3.1 To assess the impact of sirolimus on measures of reservoir size including HIV-1 DNA levels in CD4+ cells and RNA levels by conventional assay.
1.3.2 To assess the impact of sirolimus on CD4+ cell counts.
1.3.3 To assess the impact of sirolimus on HIV-1-specific CD4+ cell responses and HIV-1-specific CD8+ cell responses.
1.3.4 To assess the impact of sirolimus on T-cell activation and proliferation (% CD4+ and CD8+ cells CD38+/HLA-DR+, CD25+, PD-1+, Ki67+ and PD-L1 expression).

EXPLORATORY OBJECTIVES (NUMBERED AS IN PROTOCOL)

1.4.1 To assess the pharmacokinetic (PK) interactions between sirolimus therapy and antiretroviral (ARV) drug levels.

1.4.2 To perform ex vivo flow cytometric phenotyping of activation/proliferation in CD4+ and CD8+ central memory (CM), transitional memory (TM), effector memory (EM) and naïve populations as defined by CCR7 and CD27 expression.

1.4.3 To measure the effects of sirolimus on homeostatic proliferation, gene expression and transcriptional regulation

1.4.4 To assess the impact of sirolimus on soluble markers of inflammation, including IL-6, IL-7, and IL-15.

1.4.5 To measure the effects of sirolimus on HIV-1-specific antibody levels, the breadth of antibody responses, and B cell phenotype, rearrangements, and class switching.

1.4.6 To assess the relationship between human papillomavirus (HPV) DNA levels and HPV-related disease from anal swabs and anal Pap smears prior to and at completion of sirolimus therapy, and to assess the relationship between HPV and immune activation and reservoir size.

1.4.7 To evaluate whether the use of sirolimus is associated with more frequent reactivation or suppression of human herpes viruses (i.e. cytomegalovirus [CMV], Epstein Barr virus [EBV], herpes simplex viruses [HSV], human herpes viruses [HHV] 6, 7, and 8) as measured in longitudinally collected oral swabs.

4 ANALYSIS REPORTS

4.1 Interim Analysis Reports

Details not included.

4.2 Final Analysis Reports

The final analysis report**s** will include all sections in this analysis plan. **Because of the laboratory testing timelines, the final analysis will be provided in several separate analysis reports as data become available for analysis.**

4.3 Lists of Data in Reports

Lists of data about individual study participants may be useful for interpreting the results of the study and so some lists are described below. To protect confidentiality, the content of these lists will be limited, and for final analyses will not include dates, participant identifier numbers or other combinations of information that might identify an individual participant.

5 GENERAL ANALYSIS CONSIDERATIONS

5.1 Definition of "study entry" and "baseline"

"Study entry" is defined as the study registration date. For the purposes of statistical analysis, study week 0 will include evaluations up to and including the date of study entry. Baseline will be defined as the average of measurements from entry and week 12 (sirolimus initiation week)**, or one of these time points if the other is missing.**

5.2 Period of Follow-up

Analyses in this document address the period of follow-up defined by the protocol (44 weeks following **study** entry).

5.3 Visit Schedule and Definition of Week for Analysis Purposes

The visit schedule for key study outcomes is shown in Table 1.

The protocol requires evaluations to be scheduled at the **treatment week 0** visit, ±1 day at the **treatment week 1**, **2 and 4** visits, ±1 week at the **treatment week 8, 12 and 20** visits, and ±2 weeks at the **treatment week 32*** visit. The analysis window for post-entry visits will be calculated based on the number of **weeks** since the initial dose of sirolimus and broader windows will be used (as compared to the protocol specified window) in order to minimize the impact of deviations from the desired schedule. If there are multiple evaluations within the window for a given visit, then the evaluation closest to the scheduled study week will be used (and the later measurement will be used if there are two measurements which are equally distant from the scheduled week). Table 1 **below** details the **analysis windows for clinical data.**

For the primary and secondary outcome measures, and the exploratory soluble marker outcome, a database review was conducted by the study chair to identify the time points to be tested based on the availability of samples and the duration of sirolimus use. This resulted in a total of 159 time points for 29 study participants.

- **For 16 participants with the full 20 weeks of sirolimus use who will constitute the primary efficacy population: treatment weeks -12, 0, 4, 12, 20 and 32***
- **For the 13 participants with at least 6 but less than 20 weeks of sirolimus use: treatment weeks -12, 0, 4, 12 and 20***

  Note: One participant went off study early and only has weeks -12, 0 and 4.

Note: Treatment weeks 32* and 20* refer to time points after sirolimus discontinuation. Data from these time points will be used to assess durability of treatment effects and will not be included in the as-treated analysis of efficacy.

Table 1: Study Visit Schedule and Analysis Windows

Evaluation	Screening	Entry	Sirolimus Initiation	Post-Entry Evaluations						
	Treatment Week	**-12**	**0**	**1**	**2**	**4**	**8**	**12**	**20**	**32**
Analysis Window (**Weeks** Post-Sirolimus Initiation)			0	**(0 – 1]**	**(1 – 3]**	**(3 – 6]**	**(6 – 10]**	**(10 – 16]**	**(16 – 26]**	**(26 – 37]**
Clinical Assessment	X	X	X	X	X	X	X	X	X	X
Hematology	X	X	X	X	X	X	X	X	X	X
Liver Function Tests	X	X	X	X	X	X	X	X	X	X
Blood Chemistry	X	X	X	X	X	X	X	X	X	X
Calculated Creatinine Clearance	X	X	X	X	X	X	X	X	X	X
Urinalysis	X		X			X	X	X		
CD4+/CD8+	X	X	X		X	X	X	X	X	X
Plasma HIV-1 RNA	X	X	X			X	X	X	X	X

6 ACCRUAL

Number screened, number enrolled, number with eligibility violations and **eligibility-based** exclusions from analysis (if applicable).

Tables: Number (%) enrolled by month/year of enrollment:

Dates of first and last enrollments will be provided in a footnote to the table.

Table: Number (%) enrolled by site

7 BASELINE CHARACTERISTICS

Baseline characteristics will be presented for the full study population, as well as for the primary safety and primary efficacy analysis populations.
Tables: Showing the following variables:

Demographic and enrollment information:

a. Sex: number (%).
b. Participant reported race/ethnicity: number (%) by category used in eligibility screening.
c. Age on the day of study entry (years): N, median and 25th and 75th percentiles, min, max.
d. IV drug use: number (%) by category (never used, previously used, current user).

Health status information:

a. HIV-1 RNA (copies/mL): N, number (%) by category (<LLOQ, ≥LLOQ) (separately for week 0 and 12).
b. **Baseline** CD4+, CD8+ cell count (cells/mm^3): N, median and 25th and 75th percentiles, min, max.
c. **Baseline** CD4/CD8 ratio: N, median and 25th and 75th percentiles, min, max.
d. **Baseline** Weight (kg): N, median and 25th and 75th percentiles, min, max.
e. **Baseline** BMI (kg/m^2): N, median and 25th and 75th percentiles, min, max; number (%) by category (underweight <18.5, normal 18.5-<25, overweight 25-<30, obese ≥30).
f. Class of ARV regimen taken at entry: number (%) by category (NNRTI, non-PI, non-NNRTI, or a non-PI RPV based regimen).
g. **ARV regimen taken at entry: number (%).**

8 STUDY STATUS AND LOSS TO FOLLOW-UP

8.1 Study Status and Completeness of Follow-up of Participants

Table: Number (%) for the following categories for the overall study population:

i. Completed protocol
Note: At final analysis, participants will be considered to have completed the protocol if they have a week 44 clinic visit.

ii. "Off study" due to death.
iii. "Off study" prior to closure of study for reasons other than death (with subcategories showing reason and whether or not prior to week 12.

8.2 Duration of Follow-up Achieved

Table: Time (weeks) from study entry to last **clinic** visit reported: median, 25th and 75th percentiles, minimum, and maximum. Table will show results for the overall study population.

9 SIROLIMUS TREATMENT STATUS

Table: summarizing number (%) of participants who never started, **started and then prematurely discontinued, started and completed sirolimus treatment.**

Listing: summarizing reasons for **premature sirolimus discontinuations**

10 ANTIRETROVIRAL THERAPY STATUS

Table: summarizing number (%) of participants who have stopped ART treatment for more than 2 consecutive days, identifying whether the participant permanently discontinued or temporarily interrupted ART prior to, at, or after week 12.

11 PREGNANCY OUTCOMES (IF ANY PREGNANCIES)

List: Sirolimus dosage (if any), site, study week of pregnancy outcome, pregnancy outcome, week of last menstrual period, gestational age at pregnancy outcome, ART taken from study entry/pregnancy onset to time of pregnancy outcome (showing also week of changes in ART, from TXT0004).

List: Other pregnancy outcome information collected on form EVW0180.

12 ADVERSE EVENTS

Adverse event reporting requirements:

The protocol requires grading of events according to The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014, which is available on the RSC website: http://rsc.tech-res.com/safetyandpharmacovigilance/.

The protocol makes the following reporting requirements for signs and symptoms in section 6.3.4: "At entry, all grades of signs and symptoms that occurred within 30 days before entry must be recorded; post-entry, all grades of Grade ≥2 must be recorded. Record **on the CRF and key within 48 hours** all signs and symptoms regardless of grade that led to a change in study treatment or a change in ART. All Grade 3 or higher sign/symptom, any sign/symptom regardless of grade that led to a change in study treatment, or that met ICH, EAE or SAE guidelines, are defined by the protocol as reportable events that will require more detailed event reporting and keying within 48 hours."

The protocol makes the following reporting requirements for laboratory test abnormalities in section 6.3.5: "The screening and entry protocol-required laboratory values, regardless of grade, must be recorded. For post-entry assessments, record **on the CRF and key within 48 hours,** lipid **values**, glucose, hematology, liver function tests, calculated CrCl, and serum creatinine values regardless of grade and all other Grade ≥3 laboratory values. In addition, **record on the CRF and key within 48 hours** all laboratory values regardless of grade that led to a change in study treatment or a change in ART. All Grade 3 or higher laboratory values, any laboratory value regardless of grade that led to a change in study treatment, or that met ICH, EAE or SAE guidelines, are defined by the protocol as reportable events that will require more detailed event reporting and keying within 48 hours."

The protocol makes the following reporting requirements for diagnoses in section 6.3.4: "After entry, record all diagnoses identified by the ACTG criteria for clinical events and other diseases. Diagnoses must be recorded and keyed within 48 hours of the evaluation."

12.1 Signs/Symptoms, Abnormal Laboratory Test Values and Diagnoses

List: showing **all grades of** safety events for each study participant. The list will include the following:

- public ID
- core team assigned code (regarding the relatedness to study treatment)
- **treatment week of event**
- event description
- value and units (for laboratory results)
- grade

NOTE: This information corresponds to the ToxSum report prepared by the DMC

13 PRIMARY OUTCOME MEASURES

The primary outcome measures are defined in the protocol, section 9.2.1, as:

Safety:

Occurrence of a new Grade ≥3 AE, including signs/symptoms, lab toxicity or clinical event, that is definitely, probably or possibly related to study treatment, as judged by the core team, or by change (confirmed >50% decline or to <300 cells/mm^3) in CD4+ cell counts while on sirolimus.

Efficacy (Immunologic):

Frequency of HIV-1 Gag-specific CD8+ cells by intracellular staining for IFN-gamma at baseline and at week 32 (20 weeks on sirolimus).

Efficacy (Virologic):

CD4+ T-cell-associated HIV-1 RNA and plasma HIV-1 RNA by SCA at baseline and at week 32 (20 weeks on sirolumus).

13.1 Primary Safety Analysis

All participants who have been exposed to study treatment will be included in the **safety** analyses.

For the primary safety analysis, ≥Grade 3 AEs attributed to study treatment and confirmed CD4+ declines (see **protocol** section 9.2.1.1) will be summarized (including listing of events).

In addition, all reported AEs ≥ Grade **3** (regardless of treatment relatedness) will be summarized **(listing) and a table showing the maximum (for each participant) grade event attributed to study treatment**.

Listing**s** identifying the following:

Event description, grade, **lab value and units, treatment week of event**, and relationship to study treatment (as judged by the core team) that occurs any time from the initial dose of sirolimus to the end of study follow-up.

Similar listings restricted to lipids, glucose and creatinine, respectively.

13.2 Primary Efficacy Analyses

Because the aim of this pilot study is to investigate the biologic effects of sirolimus, the primary efficacy analysis population is defined as participants who completed the full 20 weeks of sirolimus treatment, and who remained on sirolimus and ART without virologic failure. For each primary efficacy outcome, analysis will be limited to participants who have data both at baseline and after 20 weeks of sirolimus. Based on the specimens mobilized for testing, 16 participants are anticipated to meet these criteria and be included in the primary efficacy analysis.

Tables summarizing completeness **(N)**, mean, standard deviation, median, 25^{th} and 75^{th} percentiles and min/max of HIV-1 Gag-specific CD8+ T-cells by intracellular staining for IFN-gamma, CD4+ T-cell associated HIV-1 RNA, and plasma HIV-1 RNA by SCA at baseline and at **treatment week 20**.

For the primary immunologic and virologic efficacy outcomes, post-treatment changes will be evaluated comparing measurements pre-treatment (averaged) to the measurement 20 weeks after starting study treatment, testing the null hypothesis of no difference pre-treatment versus post-treatment using a paired t-test.

Log**10**-transformations **will** be applied for the virologic outcomes.

Cell-associated HIV-1 RNA (CA-RNA) was measured in terms of copies/10^6 PBMCs. These results were normalized to copies/10^6 CD4+ T-cells by dividing by (CD4% / 100).

To address anticipated left-censoring of SCA **and CA-RNA** measurements, a value one half the lower assay limit will be imputed **and analyzed for results below assay limit**. If feasible with the modest sample size, censored-data longitudinal data methods that statistically address the issue of left-censoring (SCA measurements below assay limits) will also be used to evaluate and estimate treatment effects [Vaida and Liu 2009]. Plots will summarize the

immunologic and virologic measurements at each visit (means over time, participant-specific curves).

If there is evidence that there is a significant **effect of sirolimus on a primary immunologic or virologic efficacy outcome and also a significant** difference between the week 0 and week 12 **(treatment weeks -12 and 0)** levels of the measurements, based on paired t-tests between these two time points, then **additional analyses and summaries will be performed. In particular,** the primary analysis will **be supplemented with an analysis** based on a comparison of slopes pre- versus post-treatment. Both sets of results, from the paired t-test analyses and the comparison of slopes analyses, will be reported.

For the primary efficacy analysis population, a summary/listing of gaps or holds in sirolimus or ART use from entry through treatment week 32*.

14 SECONDARY OUTCOME MEASURES

ANALYSES:

Additional analyses of safety will summarize the magnitude of CD4+ T-cell changes baseline to post-treatment time points (means over time, participant-specific curves). Analyses will also summarize the number of **participants** who exhibit on two consecutive measurements HIV RNA >200 copies/mL on conventional assay. Tolerability will be assessed by the number of **participants** who prematurely discontinue study treatment and the reasons for discontinuation. **The analysis population for these CD4, HIV-RNA, and tolerability assessments will be the safety population.**

Analyses of secondary virologic and immunologic outcomes will parallel the primary analysis. **The secondary efficacy analysis population is defined as participants who completed at least 6 weeks of sirolimus. Paired t-tests will compare measurements pre-treatment (averaged) to the measurement at treatment week 20 for the primary efficacy population and treatment week 4 for the secondary efficacy population.** Graphical approaches will summarize the virologic and immunologic measurements over time**, through treatment week 32 for the primary efficacy population and, separately, through treatment week 4 for the secondary efficacy population**. Soluble markers (eg, IL-6 levels) will be log**10**-transformed.

Analyses will also summarize changes in immunologic and virologic measures after discontinuation of study treatment. **Paired t-tests will compare the measurements at treatment week 20 to treatment week 32* for the primary efficacy analysis population.**

For the primary analysis population, trough sirolimus levels (during the 20 weeks of treatment) will be summarized by descriptive statistics on the participant-specific average of all available sirolimus levels (minimum, 10th, 25th, median, 75th, 90th, maximum). In addition, the number of trough levels per participant will be summarized. To provide information regarding pharmacodynamics, scatterplots and correlations

will assess the relationship between average trough level and change from baseline to week 20 outcomes. These correlations will be restricted to outcomes with evidence of change following sirolimus treatment.

Associations between changes in the various immunologic and virologic measurements will be evaluated using scatterplots and correlations coefficients.

OUTCOMES:

14.1 CD4+ T-cell counts

14.2 HIV-1 RNA levels by conventional assay

14.3 HIV-1-specific CD4+ cell responses and HIV-1-specific CD8+ cell responses (other than IFN-gamma)

Measured by:

- **IFN-gamma**
- **CD107a**
- **CD40L**
- **IL-2**
- **MIP1B**
- **TNF-alpha**

14.4 T-cell activation, proliferation and subsets: % CD4+ and CD8+ cells CCR5+, CD27+, CD69+, Ki67+, PD1+, Naïve (CD45RA+CCR7+), Central Memory (CD45RA-CCR7+), Effector Memory (CD45RA-CCR7-) and Terminally Differentiated (CD45RA+CCR7-)

14.5 HIV-1 DNA levels in CD4+ cells (cell-associated HIV-1 DNA, CA-DNA)

Note: The time points for 14.1 and 14.2 will be based on the protocol visit schedule. The time points for 14.3 -14.5 will be based on the 159 selected time points for primary and secondary efficacy outcomes.

15 OTHER OUTCOME MEASURES INCLUDED IN THIS ANALYSIS PLAN

15.1 Soluble biomarkers: IL-6, IL-7, IP-10, sCD14, D-dimer (IL-15, noted in protocol, was not be tested)

15.2 T-Cell activation and proliferation (% CCR5+, CD27+, CD69+, Ki67+, PD1+) on Naïve (CD45RA+CCR7+), Central Memory (CD45RA-CCR7+), Effector Memory (CD45RA-CCR7-) and Terminally Differentiated (CD45RA+CCR7-) CD4+ and CD8+ T-cells

15.3 CD8+ T-cell counts

15.4 CA-RNA/CA-DNA Ratio

15.5 CD4+/CD8+ Ratio

15.6 CMV-specific and SEB-specific CD4+ cell responses and CD8+ cell responses,

Measured by:

- **IFN-gamma**
- **CD107a**
- **CD40L**
- **IL-2**
- **MIP1B**
- **TNF-alpha**

16 WRITING TEAM ROSTER

The writing team roster has been redacted.