A5370

Primary Statistical Analysis Plan

Version 2.0

Safety and Immunotherapeutic Activity of an Anti-PD-1 Antibody (Cemiplimab) in HIV-1-infected Participants on Suppressive cART: A Phase I/II, Double-blind, Placebo-controlled, Ascending Multiple Dose Study

ClinicalTrials.gov Identifier: NCT03787095

9/30/2020

This is ACTG A5370 SAP Version 2.0 with names of authors, names of publication writing team members and analysis timeline redacted.

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1 Introduction

1.1 Purpose

This Primary Statistical Analysis Plan (SAP) describes the primary and secondary outcome measures and additional outcome measures of the A5370 study that will be included in the primary manuscript, and which address, at a minimum, the major primary and secondary objectives of the study. The Primary SAP outlines the general statistical approaches that will be used in the analysis of the study. It has been developed to facilitate discussion of the statistical analysis components among the study team, and to provide agreement between the study team and statisticians regarding the statistical analyses to be performed and presented in the primary analysis report. It also describes the results for the primary and secondary outcome measures that will be posted on ClinicalTrials.gov.

Detailed outlines of tables, figures, and coding descriptions that will be included in the Primary Analysis Report are included in the Analysis Implementation Plan (AIP).

Analyses for the Primary Analysis Report will be finalized once the last participant of the last cohort has completed the Week 48 study visit, all queries have been resolved, and the study database closure/data lock has been completed.

Outlines of analyses for other objectives and outcome measures not included in the Primary SAP will be provided in an expanded or separate SAP for Other Objectives. Note that with the sequentially enrolled dose cohorts of the study design, laboratory assays and study analyses may commence once a cohort has fully enrolled and completed follow-up through study week 12 (see protocol section 10.1).

1.2 Key SAP Updates

Bolded text is used throughout the SAP to indicate major changes.

Version	Changes Made	Effective Date
1	Original Version	04/11/2019
1.1	No updates made after release of Protocol v2.0. Note: Protocol v2.0 is not being implemented at sites due to study closure of accrual.	12/10/2019
2.0	**Updates made to auto-immune objectives/timepoints based on Protocol version 1 LOA #2 (which added week 48 sample storage) and the team's thyroiditis additional testing plan.** **Due to early closure of the study and that not all treated participants had both infusions, efficacy analysis population modified from participants with 2**	**9/30/2020**

	infusions ≥90% to participants receiving at least 1 infusion.	

2 Study Overview

2.1 Study Design

A5370 is a phase I/II, double-blinded (within each cohort), dose-escalating, placebo-controlled study of the safety and immunotherapeutic activity of two infusions of anti-PD-1 (cemiplimab) or placebo in HIV-1-infected participants on cART who have HIV-1 RNA below the limit of quantification and CD4+ T-cell counts ≥350/mm^3. Cemiplimab (0.3 mg/kg [Cohort 1], 1 mg/kg [Cohort 2], and 3 mg/kg [Cohort 3]) or placebo will be given to 15 participants in each Cohort (12 active and 3 placebo) for a total of 45 participants. Participants will receive infusions of antibody or placebo at entry/day 0 and week 6, for a total of two infusions, with the primary safety endpoint through week 48 and key immunologic endpoints through week 12. The study will enroll sequential dose-escalating cohorts with the second and third cohorts receiving the first infusion after all participants in the previous cohort have reached week 12 and an evaluation of safety outcomes by the study team and the study monitoring committee (SMC) is completed, which determines whether to dose escalate or not.

Note: The study closed prematurely in October 2019 based on safety events observed in Cohort 1. The study will not enroll Cohorts 2 and 3 and Cohort 1 was only partially enrolled. Cohort 1 participants who received Cemiplimab will be followed for 48 weeks with frequent blood draws and safety evaluations; placebo recipients will conclude the study with a final study visit (prior to week 48).

2.2 Hypotheses

1. Two doses of the anti-PD-1 (programmed cell death) monoclonal antibody (mAb), cemiplimab, will be safe in HIV-1-infected participants on cART with plasma HIV-1 RNA suppressed below the level of quantification on standard assays.
2. Blocking PD-1 interaction with its ligands (PD-L1 and PD-L2) with anti-PD-1 antibody will enhance HIV-1-specific immune responses that promote the clearance (CL) of HIV-1-expressing cells.

2.3 Study Objectives

This Primary SAP addresses the following primary, secondary, and exploratory objectives listed in the study protocol. **In addition, two new exploratory objectives related to thyroiditis have been added by the study team based on the observed safety event.** Note: Exploratory objectives 2 and 8 are listed in this SAP for reference, but the analyses are not addressed in this document. The final analysis will occur after all participants in **Cohort 1** have completed 48 weeks of follow-up.

Analysis of the secondary study objectives below will be analyzed under a superiority framework, in particular to assess that post-treatment measures are different than pre-treatment (baseline) measures.

2.3.1 Primary Objectives

1. To assess the safety of multiple dose levels (0.3, 1, and 3 mg/kg) of cemiplimab versus placebo in HIV-1-infected, cART-suppressed participants (after 48 weeks of follow-up).

2.3.2 Secondary Objectives

1. To evaluate the change in magnitude of HIV-1 gag-specific CD8+ T cells by intracellular staining for both CD107a and interferon gamma (IFN-γ) from a pre-treatment baseline level (average of 2 measurements) to time points after one or two doses of cemiplimab (average of responses from weeks 2, 4, 6, 8, 10, and 12).
2. To evaluate the change in magnitude of HIV-1 gag-specific CD8+ T cell responses using both CD107a and IFN-γ expression from baseline to time points after the first dose of cemiplimab or placebo (average of responses from weeks 2, 4, and 6), and to time points after the second dose or placebo (average of responses from weeks 8, 10, and 12).
3. To evaluate the change in magnitude of HIV-1 gag-specific CD8+ T cells by intracellular staining for IFN-γ or CD107a alone, from baseline (pre-treatment average) to time points after one or two sequential doses of cemiplimab or placebo (average of responses from weeks 2, 4, 6, 8, 10, and 12) and explore difference following each dose.
4. To evaluate changes in polyfunctional response of HIV-1-gag-specific CD8+ T cells by intracellular staining for IFN-γ, CD107a, IL-2, and TNFα from baseline (pre-treatment average) to time points after one or two sequential doses of cemiplimab or placebo and explore difference following each dose.
5. To assess the durability of response to cemiplimab by evaluating the magnitude of HIV-1 gag-specific CD8+ T cell responses by intracellular staining of CD107a and IFN-γ and polyfunctional response through week 48.
6. To assess immunogenicity to cemiplimab after multi-dose administration to cART-treated HIV-1-infected participants.

2.3.3 Exploratory Objectives

1. To evaluate changes in total HIV-1 DNA, intact proviral HIV-1 genomes, plasma HIV RNA by single copy assay (SCA), cell-associated HIV-1 RNA, and HIV-1 RNA/DNA ratios in total CD4+ cells prior to and following cemiplimab administration.
2. To explore the human genetic and gene expression correlates of response to cemiplimab administration.
3. To explore relationships between serum cemiplimab exposure and changes in biomarkers of virologic and immune response.
4. To explore the proportion of total and HIV-1 gag-specific CD8+ and CD4+ T cells that express PD-1, PD-L1, and other exhaustion markers by multi-parameter flow cytometry. The expression profile of PD-L2 on dendritic cells and monocyte derived-macrophages will also be determined.
5. To explore changes in poly-functionality of HIV-1-specific CD4+ T cells by intracellular staining for two or more immune mediators using flow cytometry.
6. To explore changes in immune activation and cell cycling of CD8+ and CD4+ T cells by quantifying CD38+ HLA-DR+ and Ki 67 expression prior to and following anti-PD-1 administration by flow cytometry.

7. To evaluate the relationship between pre-therapy ex vivo HIV-specific T cell proliferation to HIV antigens following cemiplimab exposure and the in vivo HIV specific immune responses post cemiplimab administration.
8. To describe the pharmacokinetics (PK) and receptor occupancy (RO) of cemiplimab after administration to cART-treated HIV-1-infected participants.
9. **To explore whether there are predictors for the development of thyroiditis as an immune-related adverse event (irAE) in persons with pre-existing thyroid autoimmunity.**
10. **To describe a novel case report of the natural history of thyroiditis following administration of cemiplimab in the affected participant.**

2.4 Overview of Sample Size Considerations

Planned Study Design: The total sample size of this study is 45 evaluable participants. Within each dose cohort, there will be 12 active-treated participants and 3 placebo participants. Ultimately, there will be a total of nine placebo recipients for evaluation of immunologic and virologic outcomes. Participants who do not receive study treatment will be replaced. In addition, any participant who does not receive ≥90% of both study treatment infusions, or discontinues the study prior to week 12 without having met the primary safety endpoint (section 10.2.1.1), will be replaced. See Protocol Section 10.4 for further details on sample size calculations.

2.5 Overview of Formal Interim Monitoring

The study will undergo interim review at least annually by an independent ACTG-appointed SMC. After the last enrolled participant in each cohort receives study treatment and completes follow-up through the week 12 visit and the blinded data become available for review by the core team, safety data including the core team decision on relation of AEs to study treatment will be reviewed by the SMC, unblinded to treatment arm, to determine whether to dose escalate or modify the study. The criteria to be used to guide the SMC when determining dose resumption or dose escalation are described in Protocol Section 10.4.1.

The first review will occur 6 months after the first participant is enrolled or after all participants enrolled in cohort 1 complete 12 weeks of follow-up, whichever occurs first. Thereafter, an SMC review will occur at least annually. An interim review may also be convened if a concern is identified by the DAIDS clinical representative, the study chairs, or study statisticians in consultation with the team, or if the safety monitoring criteria in Protocol Section 10.5 are met.

3 Outcome Measures

Primary and secondary outcome measures will be included in the ClinicalTrials.gov submission one year after the PCD (48 weeks after final participant of the final cohort enrolls).

3.1 Primary Outcome Measures

1. Safety: Occurrence of a Grade ≥3 AE, or Grade ≥1 irAE (such as, but not limited to, pneumonitis, colitis, adrenal insufficiency, or hypothyroidism), that is related to study treatment (as judged by the core team, blinded to treatment arm) any time from study treatment administration through the week 48 visit.

- Aligns with Primary Objective

3.2 Secondary Outcome Measures

2. Frequency of HIV-1 gag-specific CD8+ T cells by intracellular staining for CD107a and IFN-γ at baseline and through week 12.
    - Aligns with Secondary Objective 1
3. Frequency of HIV-1 gag-specific CD8+ T cells by intracellular staining for CD107a and IFN-γ at baseline, after the first dose (average of weeks 2-6) and after the second dose (average of weeks 8-12)
    - Aligns with Secondary Objective 2
4. Frequency of HIV-1 gag-specific CD8+ T cells by intracellular staining for IFN-γ or CD107a alone at baseline and through week 12
    - Aligns with Secondary Objective 3 & 6
5. Polyfunctional response of HIV-1 gag-specific CD8+ T cells by intracellular staining for IFN-γ, CD107a, IL-2, and TNFα at baseline and through week 12
    - Aligns with Secondary Objective 4 & 6

3.3 Exploratory Outcome Measures

1. Total HIV-1 DNA, cell-associated HIV-1 RNA and RNA/DNA ratios in CD4+ T cells, and plasma HIV RNA by SCA at baseline, prior to the second infusion at week 6, and also at week 12
    - Aligns with Exploratory Objective 1
    - Virologic Measures will be log-transformed prior to analysis
2. Gene array analysis
    - Aligns with Exploratory Objective 2
3. Intact proviral HIV-1 genomes at baseline and following treatment
    - Aligns with Exploratory Objective 1
4. Ex vivo HIV-specific T cell responses following cemiplimab exposure
    - Aligns with Exploratory Objective 7
5. Expression of PD-1, PD-L1 and other exhaustion markers on CD4+ and CD8+ T cells and expression of PD-L2 on dendritic and monocyte-derived macrophages, at baseline and following treatment
    - Aligns with Exploratory Objective 4
6. Polyfunctional response of HIV-1-specific CD4+ T cells by intracellular staining at baseline and through week 12
    - Aligns with Exploratory Objective 5
7. Immune activation and cell cycling (expression of CD38/HLA-DR and Ki67 on CD4+ and CD8+ T cells) at baseline and following treatment
    - Aligns with Exploratory Objective 6
8. Levels of immunologic and virologic measures through week 48
    - Aligns with Secondary Objective 5
9. Pharmacokinetic parameters (including Cmax, t1/2) and RO of anti-PD-1 antibody following treatment
    - Aligns with Exploratory Objective 8

10. **Thyroid-stimulating antibodies, anti-thyroglobulin antibodies and anti-TPO antibodies in all participants at baseline (pre-treatment), week 6, week 16 (6 weeks post second infusion), week 24 and week 48.**
    - **Aligns with Exploratory Objective 9**
11. **Longitudinal measurement of thyroid-stimulating antibodies, anti-thyroglobulin antibodies and anti-TPO antibodies in parallel with thyroid function studies (TSH, free T4, total T3) at baseline (pre-treatment), weeks 6, 16, 24 and 48 in the participant with thyroiditis.**
    - **Aligns with Exploratory Objective 10**

4 Statistical Principles

4.1 Timeline for Analyses

Assays and statistical analyses of study data will be performed once all participants in **Cohort 1** complete the full 48 weeks of follow-up.

4.2 Lists of Data

Listings of data about individual study participants will be useful for interpreting the results of the study and so some lists will be included in the reports. To help protect confidentiality of data, the content of these lists will be limited and will not include dates, participants' study identifier numbers or other combinations of information that might identify an individual participant.

4.3 Analysis Populations

- All participants who have been exposed to the study treatment/placebo will be included in the primary safety analyses.
- Secondary efficacy analyses will be "as-treated". To maximize the ability to identify treatment effects, **given the early closure of the study,** efficacy analyses will be based on participants who **received at least one** study treatment infusion. In addition, if any participant interrupts cART for more than 7 consecutive days during the study, only measurements prior to cART interruption will be used in efficacy analyses. Participants who had virologic failure but did not interrupt cART will be included in the analysis.

4.4 Baseline Considerations

- **Baseline refers to parameters measured prior to the first receipt of study treatment.**
- Baseline measures will be the average of the pre-treatment measures**, excluding the screening measures except where the only pre-treatment measure was at screening**.
- Baseline characteristics will be summarized by arm and there will be no statistical comparisons comparing arms because of the randomized study design.

4.5 Analysis Visit Windows

- The analysis visits occur at pre-entry/entry (baseline), weeks 1, 2, 4, 6, 7, 8, 10, 12, 24, 28, 36, and 48.
- Analysis visit windows for post-baseline visits **not related to site-performed safety assessments** will be formed around each study visit using the midpoints between adjacent visits as cutoffs and potentially including assessments collected outside the recommended visit windows described in the protocol (Note: the week 6 window will not extend past the date of the second infusion). If there are multiple evaluations within the analysis window for a given visit, the evaluation closest to the scheduled study week will be used.

4.6 General Considerations

- Immunologic and virologic measures will be evaluated by comparing measurements at baseline to an average of post-treatment measurements through week 12, analyzing the actively treated participants from **Cohort 1** using the paired t-test.
    - The rationale to average over multiple post-treatment timepoints is that the timing and dynamics of treatment-induced immunologic (or virologic) effects is not known; the average of multiple time points aims to capture both early effects that may wane or, for example, a response that only appears following the second infusion.
    - **Given the early closure of the study and the small sample size available for analyses, it is anticipated that the study will only be able to identify substantive treatment effects by the t-test.**
- **Following the planned analyses, additional descriptive analyses and plots may be performed based on team discussions, for example to further examine any treated participant with an apparent immunologic response (see Gay et al., JID 2017).**
- Categorical data will be summarized using N (%), and continuous data using N, min, Q1, median, Q3, max, and mean (standard deviation (SD)) (when appropriate).
- Virologic measures such as cell-associated HIV-1 RNA will be log-transformed prior to analyses.
- **Adverse events which have a toxicity grade of 'Not Gradable' will be set numerically to '8' if necessary.**
- Missed visits will be summarized by study visit. As the endpoints average over the post-treatment measurements, no imputation will be done **for missed visits**.
- Prior to initiating analyses for an outcome, the statisticians in conjunction with the laboratory data manager and testing laboratory personnel will perform data review and QA, including assessment and confirmation regarding outliers and results below assay limits.

4.7 Analysis Approaches

4.7.1 Primary Safety Outcome

The analysis will be conducted in the safety population as defined in Section 4.**3**. AEs in the primary safety outcome are Grade ≥3 AE, or Grade ≥1 irAE (such as, but not limited to,

pneumonitis, colitis, adrenal insufficiency, or hypothyroidism), that is related to study treatment (as judged by the core team, blinded to treatment arm) any time from study treatment administration through the week 48 visit. Both primary outcome AEs and all AEs attributed to study treatment will be described separately for the treatment arm and for the placebo arm. The number of participants with a primary safety outcome will be tabulated.

4.7.2 Immunologic and Virologic Outcomes

Immunologic and virologic outcomes will be conducted in the efficacy population as defined in Section 4.**3**. Analyses will follow as described in Section 4.**6** including a paired t-test jointly analyzing actively treated participants. **Tabular** approaches will display the means at each visit and mean changes from baseline to each follow-up visit (and separately to the average of weeks 2-6 and to weeks 8-12), separately for the treated and the placebo participants. Longitudinal participant-specific plots will be presented for the treated and for the placebo participants.

Because low-level viremia as measured by SCA is anticipated to be below assay limit in a substantial fraction (~50%) of participants, binary (GEE) or censored-data longitudinal analysis approaches are planned to assess treatment effects on SCA.

The timing of treatment effects on immunologic and virologic outcome measures will be investigated by identifying, for each measure, the post-treatment timepoint with the greatest magnitude change from baseline.

The associations between ex vivo HIV-specific T-cell responses following cemiplimab exposure (based on pre-treatment samples) and in vivo HIV-specific immune responses to treatment will be evaluated using Spearman correlations. Correlation analyses will also evaluate associations between changes in the various immunologic and virologic outcomes.

4.7.3 Thyroiditis Analysis Outcomes

Measurements from the thyroiditis analysis (Exploratory Outcomes 3.3.10 and 3.3.11), which will be batch tested using stored samples, will be analyzed descriptively, summarizing the measurements at each timepoint and changes from baseline to each timepoint and with data displays using longitudinal participant-specific plots. The treated and placebo participants will be summarized and displayed separately.

Complementing these analyses, similar descriptive summaries will be presented for safety-related laboratory measures measured longitudinally by sites in the study: liver function tests (AST, ALT), TSH, free T4, fasting blood glucose, AM cortisol, free testosterone and auto-immune studies (TPO antibody, GAD65 antibody, islet cell antibody).

4.7.4 PK and RO Outcomes

Descriptive summaries, by dose group, will be presented for the estimated PK parameters (see Protocol Section 11.0). Graphical approaches will display concentration curves over time, as well as RO over time.

5 Report Contents

Detailed descriptions of the content of each of the following sections are given in the AIP. **It is anticipated that the immunologic and virologic outcomes will be presented in a separate analysis report.**

1. Study entry **information (for CONSORT)**:
    a. Screening
    b. Accrual
2. Baseline characteristics
3. Study **and treatment** status
    a. Completed study
    b. Premature study discontinuation, with reasons
    c. Completed treatment
    d. Premature study treatment discontinuation, with reasons
    e. Summary of treatment infusions
4. ART Summary
5. Virologic failure
6. HIV-1 RNA levels over time
7. CD4 **and CD8** cell counts over time
8. Safety Analyses
    a. Primary safety outcome
    b. Listing of irAE details
    c. Other AEs on study
9. Analysis of secondary outcome measures
10. **Analysis of exploratory outcome measures**