Approvals

This is ACTG A5354 SAP Version 3.0 with names of authors, names of publication writing team members and analysis timeline redacted.

ACTG 5354

Primary Statistical Analysis Plan

Version 3.0

Effect of Antiretroviral Treatment Initiated During Acute HIV-1 Infection on Measures of HIV-1 Persistence and on HIV-1-Specific Immune Responses

Protocol Version 2.0; LOA#1 and LOA#2

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Version History

Version	Changes Made	Date Finalized
1.0	Original Version	11MAY2017
2.0	Updates for protocol version 2.0 and latest CBAR SAP template	05MAY2020
3.0	Updates for LOAs to protocol version 2.0	14SEP2021

1 Introduction

1.1 Purpose

This Primary Statistical Analysis Plan (SAP) describes the primary and secondary outcome measures and additional outcome measures of the ACTG A5354 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.

A subset of these analyses (as described herein) will form the basis of reports provided to the Study Monitoring Committee (SMC) while the study is ongoing. This analysis plan therefore includes the key analyses that might lead to modification or termination of the study, and hence form the core of any presentation or publication used to disseminate the primary conclusions of the study. However, it is recognized that this analysis plan may be modified by the study team as new information becomes available outside of the study, or to reflect recommendations made by the SMC or changes in the study design. In addition, some analyses, tables, or figures may be omitted at interim analyses if there are insufficient data to warrant analysis or at the request of the SMC.

Analyses for the Primary Analysis Report will be finalized once the last participant has completed the Week 72 study visit on Step 1, all queries have been resolved, and the standard study database procedures have been completed.

Outlines of analyses for other objectives, including Step 2 follow up, and outcome measures not included in the Primary SAP will be provided in a separate SAP.

1.2 Version History

Version 2.0 of the A5354 SAP was created primarily in order to reflect changes made to the study in protocol v2.0.

One major change to the study in protocol v2.0 was an addition of 144 weeks of additional follow-up, incorporated as Step 2 of the study. Analyses for Step 2 will be encapsulated in a separate analysis plan.

Protocol v2.0 also states that the cell-associated DNA testing performed for the primary outcome measure at week 48 on Step 1 will be done separately by gag- and integrase-assays. Therefore, the analyses outlined for the primary outcome measure have been updated to reflect the pair of results that will be generated.

Version 3.0 of the A5354 SAP was updated to reflect changes to the study via LOA #1 and #2 to protocol version 2.0. Several secondary objectives and corresponding outcome measures were redesignated as exploratory objectives and ‘other’ outcome measures.

2 Study Overview

2.1 Study Design

A5354 is a phase II, prospective, open-label 2-step study to measure the effects of early antiretroviral therapy (ART) on the establishment of HIV-1 reservoir and HIV-1-specific immunity. Treatment-naïve participants with acute HIV-1 infection (AHI) will have an enrollment visit that will include the immediate initiation of ART. The primary outcome measure is cell-associated HIV-1 DNA (CAHD) in 5 million blood-derived CD4+ T-cells assayed by quantitative PCR (qPCR) at week 48 on Step 1.

A subset of participants who have completed week 48 on Step 1 will be consented for leukapheresis or large volume blood collection, cerebrospinal fluid collection via lumbar puncture, and gut biopsy via flexible sigmoidoscopy. Approximately 25 participants are estimated to have each optional procedure. Participants may consent to one or more of these optional procedures, which will be performed at any time on Step 1 from week 60 to week 72 in willing participants.

Willing participants who have completed Step 1 follow-up without meeting any criteria for permanent study discontinuation (per section 9.2) will be encouraged to continue on Step 2 and complete study evaluations through 144 weeks of Step 2 follow-up. Participants who had previously completed the study at week 72 per protocol version 1.0 will be allowed to re-enroll in protocol version 2.0 if they meet Step 2 eligibility criteria. The “re-enrolled” participants will be placed in their original Fiebig group that they were initially enrolled under protocol version 1.0

The Fiebig stage-classification system will be used to characterize the progression from HIV-1 exposure to HIV-1 seroconversion at the time of ART initiation. Plasma and serum samples will be collected at the time of ART initiation and the results of the Fiebig stage at ART initiation will be available to sites within 12 weeks. In this study, the Fiebig I-V stages of interest will be simplified into three study groups (based on HIV-1 antibody diagnostic profile at the time of ART initiation) as described below.

Group 1: Fiebig I/II (non-reactive HIV-1 antibody)

Group 2: Fiebig III/IV (reactive HIV-1 antibody and negative or indeterminate results on the Western blot or Geenius HIV-1/HIV-2)

Group 3: Fiebig V (reactive HIV-1 antibody and positive Western blot or Geenius HIV-1/HIV-2 without p31 band)

It is possible that a small number of participants will be determined to be in Fiebig VI (positive Western blot or Geenius HIV-1/HIV-2 with p31 band) based on analysis of the entry samples even though these participants are not specifically targeted for enrollment in this study. Participants who are determined to be in Fiebig VI will be followed on the study for no more than 24 weeks on Step 1, allowing ample time for them to pursue alternative sources for ART. Confirmed Fiebig VI participants will be replaced.

The Clinical Management Committee of the A5354 protocol team will review the Fiebig staging and ensure that these results are communicated to the site, as described in the study's Manual of Procedures.

2.2 Duration

Up to 216 weeks (72 weeks on Step 1 and 144 weeks on Step 2).

2.3 Sample Size

A total of 196 participants. Each study group had a target enrollment of 50 participants under protocol version 1.0. From these study groups, up to 25 eligible participants will be asked to consent to each optional procedure.

2.4 Population

Men and women 18 years and older with AHI. Pregnant and breastfeeding women are permitted to enroll in the study.

2.5 Regimen

The antiretroviral (ARV) regimen provided through the study is the single tablet regimen elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (EVG/COBI/FTC/TAF) or bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF). Other non-study-provided ARV regimens will be allowed for participants who are pregnant, breastfeeding, or unable/unwilling to take EVG/COBI/FTC/TAF or BIC/FTC/TAF, or for participants whose local health care/primary care provider prefers starting a different initial ARV regimen.

2.6 Hypotheses

Timing of antiretroviral therapy (ART) during different stages of acute HIV-1 infection (AHI) will be predictive of the magnitude and characteristics of HIV-1 reservoirs, and the quantity and quality of HIV-1-specific immune responses at week 48 on Step 1.

- ART initiation prior to HIV-1 antibody detection will be associated with limited or no detectable proviral DNA or HIV-1-specific immune responses at week 48 on Step 1.
- ART initiation in later AHI stages will be associated with larger HIV-1 reservoir establishment and quantitatively and qualitatively different HIV-1-specific immune responses at week 48 on Step 1.

2.7 Study Objectives

2.7.1 Primary Objectives

To compare the amount of cell-associated HIV-1 DNA (CAHD) in 5 million blood-derived CD4+ T-cells (assayed by quantitative PCR [qPCR]) at week 48 on Step 1 among participants who initiated ART in Fiebig I/II versus Fiebig III/IV versus Fiebig V and had sustained suppression of plasma HIV-1 RNA

2.7.2 Secondary Objectives

1. To evaluate HIV-1-specific CD4+ and CD8+ T-cells by flow cytometry while HIV-1 RNA is

suppressed on ART:

a. magnitude and distribution of CD4+ and CD8+ T-cells responses to nef, gag, pol and env
b. quality of T-cell responses (i.e. frequency and type of polyfunctional responses)

2. To assess cell-associated HIV-1 DNA in participants prior to ART initiation.
3. To establish a group of well-characterized, adults living with HIV who initiated ART during AHI that can serve as a source population for future ACTG studies, including those designed to test novel interventions to achieve HIV remission.

2.8 Overview of Sample Size Considerations

See protocol section 10.4 for details on the sample size considerations.

2.9 Overview of Formal Interim Monitoring

The study will be reviewed by a Study Monitoring Committee (SMC) according to ACTG standard operating procedures. The SMC will review accrual, baseline characteristics (including baseline laboratory values), Fiebig stage at ART initiation, AE summaries, CD4+ T-cell counts and HIV-1 RNA levels/suppression over time, study and ART discontinuations (and reasons), and completeness of follow-up and sample availability. The SMC will convene, at a minimum, 6 months after the first participant is enrolled or after 25 participants are enrolled, whichever is earlier and approximately annually thereafter or at the request of the SMC.

3 ANALYSIS REPORTS

3.1 Interim Analysis Reports

As outlined in the A5354 Study Progress, Data, and Safety Monitoring Plan (SPDSMP), an analysis report will be prepared and distributed for each SMC review. The report will be distributed to the SMC, DAIDS Clinical Representative, and the ACTG SDAC Coordinating Statistician for the SMC. The report will include analyses from the following sections of this analysis plan broken out by study group:

1. Accrual
2. Baseline characteristics
3. Fiebig stage summary (not included after accrual has completed)
4. Acute HIV-1 infection testing summary (not included after accrual has completed)
5. Study status and loss to follow-up
6. Antiretroviral treatment status
7. Adverse event summary
8. Pregnancy outcomes (if any)
9. CD4+ cell counts over time
10. Standard HIV-1 RNA copies/mL over time
11. Virologic failures
12. Summary of availability of results for primary outcome measure (not included after primary analysis is completed)
13. Summary of optional procedures performed and completed

3.2 Primary Analysis Report

Unless otherwise noted, the primary analysis report will include all sections in this analysis plan.

Section 5-8 have been summarized in a separate analysis report titled “Analysis report evaluating criteria for diagnosing acute HIV infection” (dated July 23, 2020). Unless there are additional data, analyses in these sections will not be included in the primary analysis report.

3.3 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 primary analyses will not include dates, participant identifier numbers or other combinations of information that might identify an individual participant.

4 GENERAL ANALYSIS CONSIDERATIONS

4.1 Definition of “study entry”, “baseline”, and “study group”

“Study entry” is defined as the study registration/randomization date. For the purposes of statistical analyses, study week 0 will include evaluations up to and including the date of study entry and, unless otherwise defined, baseline will be the last evaluation on or before the date of study entry.

The term ‘study group’ is used throughout this analysis plan to differentiate participants entering the study during Fiebig stages I-II (Group 1), stages III-IV (Group 2) and stage V (Group 3). At screening, a participant’s study group is estimated based on the available laboratory test results at the site. The estimated study group will be confirmed/rejected by centralized testing of samples taken at the entry visit. For interim analyses, participants who have yet to have the centralized Fiebig Stage result will be placed in a ‘Pending’ group. Once a participant’s centralized test result is available, they will then be grouped by these results. It is possible that a participant, who is estimated to be in one group at screening, will be found to be in a different group when the centralized results become available. At the primary analysis, it is intended that all participants will be grouped according to the results of the centralized testing.

Generally, tables will present results for the overall population and by study group. Select tables may also be presented by geographic region (domestic vs. international).

4.2 Period of Follow-up

Analyses in this document address the period of Step 1 follow-up defined by the protocol (up to 72 weeks following study entry). A separate analysis plan will describe the analyses for Step 2 follow-up (an additional 144 weeks following the completion of Step 1) as well as exploratory objectives.

4.3 Analysis Populations

All participants enrolled to the study will be included in accrual and centralize Fiebig result summaries.

Participants found to be HIV negative after enrollment will not be included in any summaries except those stated above. Participants found to be in Fiebig Stage VI will have baseline and select follow-up data summarized from the period of time prior to going off study (Week 24).

Analysis of the primary outcome measure will be restricted to study groups 1-3 (as determined by centralized testing), and only for participants who maintain HIV-1 RNA <50 copies/mL at week 48 with no ART interruption of ≥7 consecutive days and have available CAHD results from week 48 or week 49. Sensitivity analyses will include participants with extended periods of ART interruption (≥7 consecutive days) that maintained HIV-1 RNA <50 copies/mL at week 48.

4.4 Visit Schedule and Definition of Week for Analysis Purposes

The Step 1 visit schedule for key study outcomes is shown in Table 1 below. The protocol requires evaluations to be scheduled at week 1 (±3 days), week 4 (±7 days), week 12, 24 and 36 (±14 days), week 48 (-14 days, +3 days), *week 49 (-3 days, +14 days), and weeks 60 and 72 (±42 days).

***NOTE:** The week 49 visit is optional, in the event that blood volumes were not sufficient at the week 48 visit.

Telephone evaluations will occur at week 2 (±3 days), week 8 (±7 days) and at weeks 60 and 72 the day following performance of the gut biopsy and/or lumbar puncture optional procedures.

For the purposes of analysis, visit windows will be formed around each study visit using the midpoints between adjacent weeks as cutoffs, and potentially including assessments collected outside the recommended visit windows described above. If there are multiple evaluations within the analysis window for a given visit, the evaluation closest to the scheduled study week will be used, and the earlier measurement will be used if there are two measurements that are equally distant from the scheduled week.

Table 1 on the next page details the analysis windows for the data collected on Step 1.

Table 1: Study Visit Schedule and Analysis Windows for Step 1

Evaluation	Entry	Post-Entry Evaluations (Weeks)								
	0	1	4	12	24	36	48	49*	60	72
Analysis window		1-14 days	15-56 days	57-126 days	127-210 days	211-294 days	295-378 days		379-462 days	463-546 days
Fiebig Staging	X									
Clinical Assessment	X	X	X	X	X	X	X		X	X
Complete Physical Exam	X									
Targeted Physical Exam		X	X	X	X	X	X	X	X	X
Height	X									
Weight	X	X	X	X	X		X	X	X	X
Hematology, Chemistries, LFTs	X		X	X	X		X		X	X
CD4+/CD8+ T-cells	X			X	X		X		X	X
PBMC and Plasma Storage	X		X	X	X		X	X	X	X
Plasma HIV-1 RNA	X		X	X	X	X	X		X	X
Total HIV-1 DNA by qPCR	X						X	X		
Adherence Assessment		X	X	X	X	X	X	X	X	X
Leukapheresis (optional)	X								X	
Large Volume Blood Collection (optional)	X								X	
Gut Biopsy (optional)									X	
Lumbar Puncture (optional)									X	

*NOTE: The week 49 visit is optional, in the event that blood volumes were not sufficient at the week 48 visit.

4.5 Validation of Programs

All programs used to create datasets used for analyses detailed in this SAP will be validated following the CBAR SOP, PROG.10083, for both interim analyses for SMC review and for the primary analysis. Validation of analysis programs following CBAR SOP, PROG.10083, will be undertaken for analyses addressing the study's primary objective and key secondary objectives; these analyses are indicated in the relevant sections below.

Furthermore, per CBAR SOPs (PROG. 10083), analyses and datasets contributing to the primary objective will be independently programmed ("double coded"). This includes all analyses, outcome measures and covariates detailed in Section 17 of this document.

5 ACCRUAL

Table: Number enrolled by study group showing, if applicable, number with eligibility violations and exclusions from analysis.

List (if applicable): Description of violations of eligibility criteria, and details of exclusions from analyses (including participants who are HIV Negative and Fiebig VI).

Tables: Number (%) enrolled by month/year of enrollment: cross-tabulation by study group. Dates of first and last enrollments will be provided in a footnote to the table.

Table: Number (%) enrolled by country/site: cross-tabulation by study group.

6 BASELINE CHARACTERISTICS

Tables: Showing following variables:

Demographic and enrollment information:

a. Sex: number (%).
b. Gender identity: number (%)
c. Participant reported race and ethnicity: number (%) by category used in eligibility screening.
d. Age on the day of study entry (years): N, median and 25th and 75th percentiles, min, max; number (%) by age group (18-29, 30-39, 40-49, 50+; years, rounded down).
e. IV drug use: number (%) by category (never used, previously used, current user).
f. Self-reported risk of HIV exposure: number (%) by category (Homosexual contact, heterosexual contact, injectable drug use, unknown/not reported)

Health status information:

a. HIV-1 RNA (copies/ml): N, number (%) by category (<40, 40-<1,000; 1,000-<10,000; 10,000-<100,000; 100,000-<1,000,000; 1,000,000-<10,000,000; ≥10,000,000)
b. HIV-1 RNA (log10 copies/ml): N, mean, standard deviation, median and 25th and 75th percentiles, min, max
c. CD4+, CD8+ cell count (cells/mm^3): N, mean, standard deviation, median and 25th and 75th percentiles, min, max; number (%) by category (<50, 50-199, 200-349, 350-499, ≥500.)

d. CD4/CD8 ratio: N, mean, standard deviation, median and 25th and 75th percentiles, min, max
e. BMI (kg/m^2): N, mean, standard deviation, median and 25th and 75th percentiles, min, max; number (%) by category (underweight [<18.5]; normal [18.5-<25], overweight [25-<30]; obese [≥30])
f. HIV symptoms at AHI identification (symptomatic; asymptomatic): N, number (%)
g. Hospitalization status at AHI identification (hospitalized; not hospitalized): N, number (%)
h. Alanine Aminotransferase and Aspartate Aminotransferase (U/L): N, mean, standard deviation, median and 25th and 75th percentiles, min, max;
i. Hematocrit (%): N, mean, standard deviation, median and 25th and 75th percentiles, min, max
j. Hemoglobin (g/dL): N, mean, standard deviation, median and 25th and 75th percentiles, min, max
k. Platelets (cells/mm^3): N, mean, standard deviation, median and 25th and 75th percentiles, min, max
l. Leukocytes (cells/mm^3): N, mean, standard deviation, median and 25th and 75th percentiles, min, max
m. Creatinine clearance (mL/min): N, mean, standard deviation, median and 25th and 75th percentiles, min, max

For Females:

n. Pregnancy test result (positive, negative, not of reproductive potential): N, number (%)
o. Breast-feeding status (no, yes): N, number (%)

For individuals with genotype results reported from SOC:

p. HIV subtype (B, C, CRF#, etc.): N, number (%)
q. HIV genotype drug resistance interpretations (Susceptible, Intermediate, Resistant, Not Reported) for each of the drugs reported: N, number (%)

NOTE: Due to the variety of genotype results reported, interpretations for a given drug may not be available for all participants

7 FIEBIG STAGE

Tables showing the following:

a. Cross tabulation of the study group estimated at entry, and that determined by centralized testing: number (%)
b. Time (weeks) between study entry and Fiebig stage results (latest assay date) determined by centralized testing: N, mean, standard deviation, median and 25th and 75th percentiles
c. Time (weeks) between screening and study entry: N, mean, standard deviation, median and 25th and 75th percentiles

d. Fiebig stage determined by centralized testing: number (%) overall and by country

e. Listing: Participants found to be HIV negative after enrollment, showing estimated Fiebig group at screening, screening assay timing/results, centralized assay timing/results, components and duration of ART regimen taken (if applicable), HIV-1 RNA assay results, site, and duration on study

8 ACUTE HIV-1 INFECTION (AHI) TESTING

This section has been defined in a separate statistical analysis plan (A5354_AHIDiag_Statistical_Analysis_Plan_v1.0_6MAR2020, dated Mar. 6, 2020).

9 STUDY STATUS AND LOSS TO FOLLOW-UP

CONSORT-style diagrams will be developed for the primary analysis summarizing key components of the study follow-up of the study population.

9.1 Study Status and Completeness of Follow-up of Participants

Table: Number (%) for the following categories:

i. 'Completed Step 1 as defined by the protocol' or 'On study' (for interim analyses).

ii. "Off study" due to death.

iii. "Off study" prior to end of Step 1 follow-up (or, at interim analyses, prior to data retrieval date) for reasons other than death (with subcategories showing reason and whether or not prior to week 48 on Step 1).

Listing: Showing all premature discontinuations with centralized Fiebig Stage result, site, timing and reason of premature discontinuation, week of last clinic visit, ARV regimen taken, and whether a sample was collected at the week 48/49 visit.

9.2 Duration of Follow-up Achieved

Table: Time (weeks) from study entry to last visit reported: median, 25th and 75th percentiles, minimum, and maximum.

NOTE: At the primary analysis, all analyses from here onwards will be undertaken after any exclusions from analyses. Participants who are determined to be Fiebig Stage VI by centralized testing will be followed and presented until they are taken off study at week 24.

10 PREGNANCY OUTCOMES

Listing: Site, study week of pregnancy outcome, pregnancy outcome, estimated week of conception, gestational age at pregnancy outcome, ART taken from study entry/pregnancy onset to time of pregnancy outcome (showing also week of changes in ART, if any).

Listing (if available): Other pregnancy outcome information collected.

11 ANTIRETROVIRAL THERAPY STATUS

Table: summarizing the ART regimen initiated at entry: number (%) [may be included in baseline table]

Table: summarizing number (%) of participants who have stopped ART treatment (No holds ≥7 days; Hold ≥7 consecutive days), reason(s) for stopping, and whether the participant permanently discontinued or temporarily interrupted ART prior to, at, or after week 48.

Table: summarizing the reasons (if any) for participants that modified their ARV regimen, along with the timing and reason for the change.

NOTE: Participants who interrupt ART for ≥7 consecutive days will be considered for withdrawal from the study. If the ART interruption occurs prior to study week 24 on Step 1 and the participant has not previously demonstrated an HIV-1 RNA <50 copies/mL (prior to the hold lasting ≥7 consecutive days), then the participant will be withdrawn from the study and undergo the premature study discontinuation evaluation.

12 ADVERSE EVENTS

The protocol requires grading of events according to The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), corrected Version 2.1, July 2017, which can be found on the DAIDS RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables.

The protocol makes the following reporting requirements for signs and symptoms:

> "At Step 1 entry, signs and symptoms of all grades that occurred within 30 days prior to entry must be recorded as medical history. Post-entry Grade ≥3 signs and symptoms and all signs and symptoms that led to a change in ART, regardless of grade"

The protocol makes the following reporting requirements for laboratory test abnormalities:

> "At Step 1 entry, record all protocol-required laboratory values (performed by local laboratories) with exception of the urinalysis, regardless of grade. For post-entry assessments, record all laboratory values for creatinine, CrCl, AST (SGOT), and ALT (SGPT) regardless of grade. Post-entry Grade ≥ 2 laboratory findings and all laboratory findings that led to a change in ART, regardless of grade"
>
> NOTE: Creatinine clearance should be graded using the categorical mL/min values from the DAIDS 2.1 toxicity grading tables, which begins with values below 90 mL/min. The percent change criteria will not apply to A5354. In Step 2, only creatinine clearance greater than Grade 2 should be reported.

The protocol makes the following reporting requirements for diagnoses:

> "At Step 1 entry, all diagnoses that are described in protocol section 6.3.4 (Acute HIV-1, bone fractures, coronary heart disease, cancer, diabetes, tuberculosis, chronic hepatitis B or C, renal disease) must be recorded as medical history. Post-entry, record all targeted diagnoses (see list on the A5354 PSWP) and diagnoses that led to a change in ART.

NOTE: Uterine pregnancy does not require reporting as an AE (unless other SAE criteria are met) but must still be recorded in the eCRFs."

Additionally, all AEs meeting the SAE definition or EAE reporting requirements, or led to a change in study treatment, must be reported.

For the purpose of ongoing safety monitoring occurring within A5354, the following data summaries will be provided at each interim analysis, separately for Step 1 and Step 2:

Table: summarizing number (%) of participants and the adverse events reported in the database, by MedDRA SOC, PT, grade and study group.

Table: summarizing number (%) of participants and the serious adverse events reported in the database, by MedDRA SOC, PT, grade and study group.

Note: For participants enrolled to Step 2, AEs on Step 1 will be summarized up to last study visit on Step 1 in the primary analysis report of Step 1. Any AEs reported after the last visit on Step 1 prior to entry to Step 2 will be summarized in the analysis for Step 2 long-term follow-up.

13 CD4/CD8 CELL COUNTS

Summaries at each measurement week:

Table: CD4+, CD8+ cell count (cells/mm^3): N, mean, standard deviation, median and 25th and 75th percentiles, min, max;

Table: Change from baseline (calculated as FU-BL) in CD4+, CD8+ cell count (cells/mm^3): N, mean, standard deviation, median and 25th and 75th percentiles, min, max

Table: CD4/CD8 ratio: N, mean, standard deviation, median and 25th and 75th percentiles, min, max.

Table: Fold change from baseline (calculated as FU/BL) in CD4/CD8 ratio: N, mean, standard deviation, median and 25th and 75th percentiles, min, max

14 STANDARD HIV-1 RNA AND VIROLOGIC FAILURE

Summaries of HIV-1 RNA at each measurement week:

Table: HIV-1 RNA viral load (log10 copies/mL): N, mean, standard deviation, median and 25th and 75th percentiles, min, max; number (%) by category (<50, ≥50; and separately for <200 copies/mL and ≥200 copies/mL)

Table: 95% confidence interval based on exact binomial distribution on proportion of HIV-1 RNA < 50 or 200 copies/mL at each study week.

Plot: Proportion of participants with HIV-1 RNA <50 or 200 copies/mL by study week, overall and by Fiebig group

Summaries/Listing of confirmed virologic failure:

Table: Number of participants with confirmed virologic failure (defined as two consecutive HIV-1 RNA >200 copies/mL at Step 1 week 24 or later or at any time after achieving HIV-1 RNA <50 copies/mL);

Listing: (For participants with confirmed virologic failure) Initial failure week and HIV-1 RNA copies/mL, confirmed failure week and HIV-1 RNA copies/mL, ART regimen at the time of initial/confirmed failure, duration of ART regimen at the time of failure, Fiebig stage/study group

15 PROCEDURE SUMMARY (INTERIM)

Table summarizing:

a. Number (%) of participants who consented and successfully completed each optional procedure
b. Number (%) of participants consenting to and receiving multiple procedures
c. Summary of week the procedure(s) was consented to and week the procedure was performed: N, median, 25th and 75th percentiles, min, max

16 DATA COMPLETENESS SUMMARY (INTERIM)

To be generated and provided by Frontier Sciences, the Data Management Center.

Will contain summaries of completeness of collection for following study data:

a. Serum creatinine, creatinine clearance
b. AST, ALT
c. CD4+/CD8+
d. Plasma HIV-1 RNA

Specimens for:

e. Fiebig Staging
f. HIV-1 Genotype
g. Total HIV-1 DNA by qPCR
h. PBMC and Plasma Storage [only if large volume blood draw or leukapheresis are not done]
i. Large Volume Blood Collection [as an alternative to leukapheresis]
j. Leukapheresis [optional procedure]
k. Gut biopsy [optional procedure]
l. Lumbar Puncture [optional procedure]

NOTE: Routine PBMC and plasma storage will be obtained from the large volume blood collection (or optional leukapheresis) at entry. Stored PBMC and plasma will be obtained at weeks 60 and 72, only if optional leukapheresis or large volume blood collection is not performed at those visits.

17 PRIMARY OUTCOME MEASURE

The primary outcome measure defined in the protocol, section 10.2.1:

> *Cell-associated HIV-1 DNA (CAHD) in 5 million blood-derived CD4+ T-cells (assayed by quantitative PCR [qPCR]) at week 48 on Step 1*
>
> ***Note: Derivation of the primary outcome measures and any contributing datasets will be double-coded in accordance with the CBAR SOP, PROG.10083.***

For interim analyses, a summary of the availability of CA-DNA results will be generated.

Table: Number (%) of participants with a week 48 visit on Step 1; Number (%) of participants with available CAHD results at week 48 on Step 1; Number (%) of participants not reaching week 48 on Step 1

17.1 Primary Efficacy Analyses

Note: Programs related to the analysis of the primary outcome measures listed in this section will require validation in accordance with the CBAR SOP, PROG.10083, including independent verification of results

A table summarizing completeness of the primary outcome measure, and reasons for missing data (if any) will be provided, overall and by study group.

Analysis of the primary outcome measure will be restricted to participants who maintain HIV-1 RNA <50 copies/mL at week 48 with no ART interruption of ≥7 consecutive days and have available CAHD results from week 48 or week 49. Specifically, plasma HIV-1 RNA by commercial assay must be <50 copies/mL at week 48 with no prior virologic failure (defined as having two consecutive HIV-1 RNA >200 copies/mL at week 24 or later or at any time after achieving HIV-1 RNA <50 copies/mL). This study is designed to evaluate the effect of suppressive ART initiated in different stages of AHI on the establishment and persistence of HIV-1 reservoirs; this is the rationale for analyzing HIV-1 DNA only among virally suppressed participants and not in those with incompletely suppressed viremia (≥50 copies/ml).

Participants who are excluded from the primary analysis will be presented in a listing, which displays the criteria by which they were excluded.

The primary outcome measure will be summarized as a binary outcome, [0 copies] versus [>0 copies] of CAHD per 5 million CD4+ T-cells, assessed separately and jointly by gag- and integrase-assays. In order for a participant to be considered as having 0 copies of CAHD, the participant must be found to have an undetectable CAHD result from both assays. The proportion of participants with ‘0 copies of CAHD’ will be compared pairwise between groups using Fisher’s exact test. A 95% confidence interval for the proportion estimated will be calculated using the exact binomial distribution. The analysis groups will be the study groups: Group 1 (Fiebig I/II), Group 2 (Fiebig III/IV), and Group 3 (Fiebig V), as defined by the results of the centralized testing from samples at study entry.

CAHD per million CD4+ T-cells will also be summarized as a continuous variable, separately for gag- and integrase-assays, presenting N, median, Q1 and Q3 for the raw CAHD values (by study group and separately by Fiebig Stage). CAHD per 5 million CD4+ T-cells will be divided

by 5 in order to evaluate CAHD per million CD4+ T-cells. Undetectable CAHD results will be imputed as 0.01 copies. Additionally, summaries will be provided for log10-transformed CAHD per million CD4+ T-cells for gag- and integrase-assays, which will present N, mean, standard deviation, median, Q1, Q3 and min/max. Plots showing median, Q1 and Q3 values over time will also be presented, overall and by study group. In addition, scatter plots of gag- vs. integrase-assays will be presented with study groups marked with separate symbols.

A supplemental analysis will use Wilcoxon rank sum test to compare the primary outcome measure on the continuous scale, separately for gag- and integrase-assays, between pairwise study groups (and between select Fiebig stages). An additional supplemental analysis will use the Jonckheere–Terpstra test to assess potential trends across the study groups (and between Fiebig stages). We will also examine the primary outcome measure that includes participants with extended periods of ART interruption (≥7 consecutive days) who maintained HIV-1 RNA <50 copies/mL at week 48. For clades with sufficient data, supplemental sensitivity analyses will compare Fiebig stages stratified by HIV subtype.

18 SECONDARY OUTCOME MEASURES

Analysis of secondary outcome measures will use the same approach as the primary outcome measure if the outcome is binary. Wilcoxon rank-sum tests will be used to compare the continuous secondary outcome measures between groups. Jonckheere-Terpstra tests will be used to assess trends across the groups. Rank-based Spearman correlations will describe association between outcome measures. Scatter plots may also be used to present associations between outcome measures. Plots showing median, Q1 and Q3 values over time will also be presented, overall and by study group.

18.1 HIV-1-specific CD4+ and CD8+ T-cell responses to nef, gag, pol and env by flow cytometry while HIV-1 RNA is suppressed on ART

The following HIV-1-specific responses will be summarized:

a. Percent of CD4(8) cells expressing each of the 5 cytokines (CD40L, CD107a, IFNg, MIP1B, TNFa) in responses to each of the 4 protein stimulants (ENV, GAG, NEF and POL)

   Parent: CD3+CD4+ or CD3+CD8+

   Cytokine Markers: CD40L+; CD107a+; IFNg+; MIP1B+; TNFa+ each individually

b. Percent of CD4(8) cells expressing any cytokine (total response) to each of the 4 protein stimulants

   Parent: CD3+CD4+ or CD3+CD8+

   Cytokine Marker: CD40L+ or CD107a+ or IFNg+ or MIP1B+ or TNFa+

c. Relative proportion of CD4(8) cells co-expressing any 2/3/4/5 cytokines (polyfunctional responses) to each of the 4 protein stimulants

Parent: CD3+CD4+Cytokine+ or CD3+CD8+Cytokine+

For each n-cytokine polyfunctional response, the response is calculated as the total of the relative percent of cells expressing exactly n cytokines for the various combinations. For example, for 4-cytokine polyfunctional response, the various combination of the 4 positive cytokines are:

CD40L-;CD107a+;IFNg+;MIP1B+;TNFa+

CD40L+;CD107a-;IFNg+;MIP1B+;TNFa+

CD40L+;CD107a+;IFNg-;MIP1B+;TNFa+

CD40L+;CD107a+;IFNg+;MIP1B-;TNFa+

CD40L+;CD107a+;IFNg+;MIP1B+;TNFa-

And similarly, for 3-cytokine polyfunctional response, there are 10 various combinations with 3 positive cytokines.

Note:

For response a) and b) above, the results for a specific outcome for a specific participant/specimen-date are calculated by subtracting the corresponding background control value (media control). If the result would be less than zero after background subtraction, the result is set to zero. If the result of the active control SEB is less than the result of the background control for a particular outcome, the observation for that participant/specimen-date is treated as no result generated and is not included in the statistical analyses or plots.

For response c), since the percentage is among cells which expressing at least one cytokine, there is no need to subtract the corresponding background control value. However, if the result of the active control SEB is less than the result of the background control for any cytokine response (response a above), then the observations with the parent CD3+CD4(8)+cytokine+ are treated as no result generated and is not include in the calculation of response c.

18.2 Cell-associated HIV-1 DNA in participants prior to ART initiation

Pre-ART cell-associated HIV-1 DNA will be summarized using the same approach as week 48 CAHD.

Fold-changes (absolute change in the log10-tranformed measurements) from pre-ART measurement in CAHD will be summarized using a similar approach.

Correlations between selected immunology responses (IFNg+ and total response) and week 48 CAHD will be examined using Spearman correlation coefficient (primary analysis population) with and without controlling for the study group.

19 OTHER OUTCOME MEASURES

Analysis of other outcome measures will use the same approach as the secondary outcome measure. The following exploratory outcome measure(s) will be included in the primary analysis report:

19.1 Signs and symptoms of acute retroviral syndrome

Table: summarizing number (%) of participants and the signs and symptoms of acute retroviral syndrome reported in the database, by MedDRA SOC, PT, grade and study group.

19.2 Absolute CD4+ and CD8+ T-cell counts, CD4/CD8 ratio, and HIV-1 RNA prior to and after ART initiation

See section 13.

Other outcome measures from the protocol not listed here will be included in a separate report.

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