A5354

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

A Limited-Center Trial of the AIDS Clinical Trials Group (ACTG)

NIAID CRMS # 12056

This file contains the current ACTG A5324 protocol:

- Letter of Amendment #2, dated 17May2021
- Letter of Amendment #1, dated 05Aug2020
- Clarification Memorandum #1, dated 14Apr2020
- Protocol Version 2.0, dated 31Dec2019

Letter of Amendment #2 for:

A5354

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

A Limited-Center Trial of the AIDS Clinical Trials Group (ACTG)

NIAID CRMS # 12056

Letter of Amendment Date: 17May2021

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LETTER OF AMENDMENT #2

DATE: May 17, 2021

TO: ACTG CTU Principal Investigators, CRS Leaders, and CTU/CRS Coordinators

FROM: A5354 Protocol Team

SUBJECT: Letter of Amendment #2 for Protocol A5354

The following information affects the A5354 study and must be forwarded to your institutional review board (IRB)/ethics committee (EC) as soon as possible for their information and review. This Letter of Amendment (LOA) must be approved by your IRB/EC before implementation.

The following information may also affect the Sample Informed Consent. Your IRB/EC is responsible for determining the process of informing participants of the contents of this LOA.

Upon receiving final IRB/EC and any other applicable regulatory entity approvals for this LOA, sites should implement the LOA immediately. Sites are still required to submit an LOA registration packet to the DAIDS Protocol Registration Office (PRO) at the Regulatory Support Center. A Protocol Signature Page (PSP) is appended for submission to the DAIDS Protocol Registration System (DPRS) as part of the LOA registration packet. Sites will receive a registration notification for the LOA once the DAIDS PRO

verifies that all required LOA registration documents have been received and are complete. An LOA registration notification from the DAIDS PRO is not required prior to implementing the LOA. A copy of the LOA registration notification, along with this letter and any IRB/EC correspondence, should be retained in the site's regulatory file.

This LOA is being implemented for the following reasons:

- revise the lists of secondary and exploratory objectives and their related outcome objectives (items #2-5)
- correct a clerical error in A5354v2 LOA#1, dated 08/05/20 (item #1)
- incorporate DAIDS template language on the possibility for remote monitoring (item #6)
- reconcile the Step 2 blood volume amounts in the Sample Informed Consent of A5354v2, dated 12/31/19 (item #7).

The following are changes (noted in bold or strikethrough) to Protocol A5354, Version 2.0, 12/31/19, titled "Effect of Antiretroviral Treatment Initiated During Acute HIV-1 Infection on Measures of HIV-1 Persistence and on HIV-1-Specific Immune Responses." These changes will be included in the next version of the A5354 protocol if it is amended at a future date.

TABLE OF CONTENTS

1. Clarification Memo

   In the A5354v2 LOA#1 (dated 08/05/20), the mentioned clarification memo (CM) dated 04/14/20 pertains to CM#1 (not CM#2 as referenced in the 2nd bullet of the introductory paragraph on page 1).

2. Secondary Objectives

   In section 1.3, Secondary Objectives, the following modifications are being made, and secondary objective 1.3.4 is being renumbered as 1.3.3.

   1.3.1 To evaluate HIV-1-specific CD4+ and CD8+ T-cells by flow cytometry ~~prior to ART initiation and~~ while HIV-1 RNA is suppressed on ART:
   a. magnitude and distribution of CD4+ and CD8+ T-cells responses to nef~~/tat/rev/vpr/vpu~~, gag, pol and env
   b. quality of T-cell responses (i.e., frequency and type of polyfunctional responses)

   ~~1.3.2 To assess the amount of unspliced HIV-1 RNA in 5 million blood-derived CD4+ T-cells prior to ART initiation and while HIV-1 RNA is suppressed on ART.~~

   ~~1.3.3~~ 1.3.**2** To assess cell-associated HIV-1 ~~RNA to~~ DNA ~~ratio~~ in participants ~~with quantifiable HIV-1 DNA~~ prior to ART initiation ~~and while HIV-1 RNA is suppressed on ART~~.

3. Exploratory Objectives

   In section 1.4, Exploratory Objectives, the objectives listed below are being added.

   1.4.10 To assess the amount of unspliced HIV-1 RNA in blood-derived CD4+ T- cells prior to and after ART initiation, including while HIV-1 RNA is suppressed on ART.

   1.4.11 To evaluate HIV-1-specific CD4+ and CD8+ T-cells by flow cytometry prior to ART initiation:
   a. magnitude and distribution of CD4+ and CD8+ T-cells responses to major structural and/or accessory HIV-1 proteins, such as nef, gag, pol, and env
   b. quality of T-cell responses (i.e., frequency and type of polyfunctional responses)

   1.4.12 To assess cell-associated HIV-1 RNA in participants prior to and after ART initiation.

4. Secondary Outcome Measures

   In section 10.2.2, Secondary Outcome Measures, the modifications shown below are being made.

10.2.2.1 HIV-1-specific CD4+ and CD8+ T-cell responses to nef~~/tat/rev/vpr/vpu~~, gag, pol, and env by flow cytometry ~~prior to ART initiation and~~ while HIV-1 RNA is suppressed on ART

~~10.2.2.2 Cell-associated HIV-1 RNA per 5 million CD4+ T-cells prior to ART initiation and while HIV-1 RNA is suppressed on ART~~

~~10.2.2.3~~ 10.2.2.**2** Cell-associated HIV-1 ~~RNA/~~DNA ~~ratio~~ in participants ~~with detectable CAHD~~ prior to ART initiation ~~and while HIV-1 RNA is suppressed on ART~~

5. Other Outcome Measures

In section 10.2.3, Other Outcome Measures, the measures shown below are being added.

10.2.3.7 HIV-1-specific CD4+ and CD8+ T-cell responses to nef, gag, pol, and env by flow cytometry prior to and after ART initiation

10.2.3.8 Unspliced HIV-1 RNA per 5 million CD4+ T-cells prior to and after ART initiation

10.2.3.9 Cell-associated HIV-1 RNA prior to ART and after initiation.

10.2.3.10 Signs and symptoms of acute retroviral syndrome

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

6. Clinical Site Monitoring and Record Availability

In section 12.3, Clinical Site Monitoring and Record Availability, the remote monitoring language and reference listed below are being added to follow the first paragraph of subsection 12.3.1.

12.3.1 Site monitors under contract to the NIAID will visit participating clinical research sites to review the individual participant records, including consent forms, eCRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts), to ensure protection of study participants, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect sites' regulatory files to ensure that regulatory requirements are being followed and sites' pharmacies to review product storage and management.

Monitoring visits may be conducted on-site or remotely. Remote visits may include remote source document verification using methods specified for this purpose by NIAID. Remote monitoring visits may be performed in place of, or in addition to onsite visits to ensure the safety of study participants and data integrity [85]. The site will make available study documents for site monitors to review utilizing a secure platform that is HIPAA and 21 CFR Part 11 compliant. Potential platform options include: Veeva SiteVault, site-controlled SharePoint or cloud-based portal, direct access to Electronic

Medical Record (EMR), and Medidata Rave Imaging Solution. Other secure platforms that are 21 CFR Part 11 compliant may be utilized, as allowed by the DAIDS Office of Clinical Site Oversight (OCSO).
FDA Guidance on Conduct of Clinical Trials of Medical Products During the COVID-19 Public Health Emergency: Guidance for Industry, Investigators, and Institutional Review Boards, March 2020, Updated on January 27, 2021. Accessed at: https://www.fda.gov/media/136238/download

7. Part 2 Study Schedule of the Sample Informed Consent of A5354v2

In the Study Schedule for Part 2 of the Sample Informed Consent of A5354v2 (dated 12/31/19), the approximate amount of blood to be collected at Step 2 entry and every 24 weeks until week 144 has been corrected to be consistent with the Revised Laboratory Processing Chart (revised date 10/23/20).

II. Study Schedule for Part 2

Evaluation or Procedure	Step 2 Entry[2]	Every 24 weeks until week 144[3]	Special Visit[5]	Leaving or Stopping the Study Early[6]
Physical exam	✓	✓		✓
ART continued	✓	✓		
ART modifications	✓	✓	✓	✓
Blood collected	✓	✓	✓	✓
Pregnancy test	✓	If suspected		✓
Adherence support	✓	✓	✓	
Approximate amount of blood	~~up to 83 mL~~ **23 mL**	up to ~~83~~ **19** mL/visit	14 mL	19 mL

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

PROTOCOL SIGNATURE PAGE

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable US Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

Principal Investigator: _____
Print/Type

Signed: _____Date: _____
Name/Title

Letter of Amendment #1 for:

A5354

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

A Limited-Center Trial of the AIDS Clinical Trials Group (ACTG)

NIAID CRMS # 12056

Letter of Amendment Date: 05Aug2020

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LETTER OF AMENDMENT

DATE: August 5, 2020

TO: ACTG CTU Principal Investigators, CRS Leaders, and CTU/CRS Coordinators

FROM: A5354 Protocol Team

SUBJECT: Letter of Amendment #1 for Protocol A5354 Version 2.0

The following information affects the A5354 study and must be forwarded to each site's institutional review board (IRB)/ethics committee (EC) as soon as possible for their information and review. This Letter of Amendment (LOA) must be approved by the site IRB/EC before implementation.

The following information may also affect the Sample Informed Consent. The site IRB/EC is responsible for determining the process of informing participants of the contents of this LOA.

Upon receiving final IRB/EC and any other applicable regulatory entity approvals for this LOA, sites should implement the LOA immediately. Sites are still required to submit an LOA registration packet to the DAIDS Protocol Registration Office (PRO) at the Regulatory Support Center. Sites will receive a registration notification for the LOA once the DAIDS PRO verifies that all required LOA registration documents have been received and are complete. An LOA registration notification from the DAIDS PRO is not required prior to implementing the LOA. A copy of the LOA registration notification, along with this letter and any IRB/EC correspondence, should be retained in the site's regulatory file.

This LOA is being implemented for the following reasons:

- A team decision has been made that participants who have previously completed the final study visit (week 72) and were discontinued from the study under Final Version 1.0 should now be contacted and scheduled for screening in Step 2 of the Final Version 2.0. Step 2 of the Final Version 2.0 is open to all participants who are currently in follow-up or who previously completed the study under Final Version 1.0 and meet Step 2 criteria.
- A team decision has been made to include instructions from A5354v2 Clarification Memo (CM #2, dated 04/14/20) related to study visits that could not be conducted in person due to SARS-CoV-2 pandemic (items #4 and #5 below).
- A team decision has been made to include a new inclusion and exclusion criteria in Step 2 (items #2 and #3 below).
- A team decision has been made to update the ARV interruption exclusion criterion for Step 2 (item #3 below).

TABLE OF CONTENTS

The following are changes (noted in bold or strikethrough) to Protocol A5354, Version 2.0, 12/31/19, titled "Effect of Antiretroviral Treatment Initiated During Acute HIV-1 Infection on Measures of HIV-1 Persistence and on HIV-1-Specific Immune Responses." These changes will be included in the next version of the A5354 protocol if it is amended at a future date. Changes that have already been made (either by Letter of Amendment or by Clarification Memo) have been incorporated in the excerpted text shown below (and are no longer presented in bold or strikethrough).

1. Protocol Signature Page (PSP)
   A Protocol Signature Page (PSP) was appended for submission to DAIDS Protocol Registration System (DPRS) as part of the LOA registration packet.

2. Section 4.3, Step 2 Inclusion Criteria
   Updated to include a new inclusion as follows. All subsequent inclusion criteria were renumbered.

   4.3.2 At least one documented HIV-1 RNA result in each calendar year since Step 1 enrollment.

3. Section 4.4, Step 2 Exclusion Criteria
   a. Updated exclusion criterion as follows.

   4.4.1 ARV interruption for greater than or equal to 7 consecutive days at any time ~~after ART initiation and prior to entry into Step 2, including while on Step 1 or~~ after completion of Step 1 **and before initiation of Step 2**.

   b. Included a new exclusion criterion as follows.

   4.4.7 Any HIV-1 RNA >200 copies/mL after completion of Step 1 and before initiation of Step 2.

4. Section 5.1, Regimens, Administration, and Duration
   Included the clarifications from A5354v2 CM#1 (dated 04/14/20) as follows.

   Study treatment is defined as STR elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (EVG/COBI/FTC/TAF) or bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF), which will be provided through the study.

   NOTES: Switching of ARV regimen is allowed (i.e., from EVG/COBI/FTC/TAF to BIC/FTC/TAF, or vice versa, or to an alternate non-study-provided ART) without consulting the CMC. Sites should ensure that participants who are enrolled into Step 2 have an adequate supply of study-provided drug (i.e., up to a 3-month provision) until a participant is able to come to the clinic for a study visit. If a participant is not able to return to the clinic for any reason and needs a refill of study-provided drug, sites must contact their protocol team pharmacist (via the Clinical Management Committee (CMC) at actg.cmcA5354@fstrf.org) to establish the procedures for shipping study-provided drug to the participant.

5. Section 6.3.3, Post-Entry Evaluations
   a. Included the notation as follows.

Step 2 Evaluations

The Step 2 Registration evaluation may be combined with the Step 1 Week 72 visit. If the Step 2 Registration visit is not combined, then all Step 2 evaluations must be performed within 2 weeks after the final Step 1 visit. Those participants re-enrolling into the study after going off study at week 72 under protocol Version 1.0 will complete all evaluations listed in the Step 2 Registration column.

NOTES: If a participant is unable to come to the clinic for the Step 2 registration visit for any of the reasons outlined in the subsection paragraphs, Remote Data Collection, of section 6.3.3, sites are encouraged to perform an alternative consenting process (e.g., telephone, telehealth). Sites should inform their IRB about this alternate consent method for Step 2.

All visits following Step 2 registration will have a ± 60-day visit window.

b. Included the clarifications from A5354v2 CM#1 (dated 04/14/20) as follows. Newly added text are in bold. The subsection entitled, Remote Data Collection, will be incorporated at the end of section 6.3.3, Post-Entry Evaluations.

Remote Data Collection

It is preferable for study visits, as outlined in sections 6.1 and 6.2 Schedule of Evaluations (SOE), to be conducted in person. Study visits may be conducted remotely (e.g., telephone, telehealth) in the following situations:

- A participant is unable to attend a visit because of personal illness, illness among others in his or her home, or local conditions or guidelines restricting travel to the clinic.
- The site is temporarily unable to conduct non-essential visits in the clinic; the site must inform the CMC when it has to stop non-essential visits.

Regardless of the situation, sites should document which visits were conducted remotely, attempt to obtain as much of the visit-specific required information, based on the SOE, as possible, and record it on the relevant eCRF. The impacted visits and rationale must be reported and documented, following instructions provided by the team or network leadership.

Implementation of remote data collection is expected to be time-limited in relation to the SARS-CoV-2 pandemic. In consultation with ACTG Network leadership and the study sponsor, the protocol team will determine when remote data collection is no longer permitted. When such a determination is made, study sites will be formally notified and instructed to inform their IRBs/ECs as needed.

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

SIGNATURE PAGE

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable US Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

Principal Investigator: _____
Print/Type

Signed: _____Date: _____
Name/Title

Clarification Memo #1 for:

A5354

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

A Limited-Center Trial of the AIDS Clinical Trials Group (ACTG)

NIAID CRMS # 12056

Clarification Memo Date: 14Apr2020

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CLARIFICATION MEMO

DATE: April 14, 2020

TO: ACTG CTU Principal Investigators, CRS Leaders, and CTU/CRS Coordinators

FROM: A5354 Protocol Team

SUBJECT: Clarification Memo #1 for A5354 Protocol Version 2.0 (SARS-CoV-2 Pandemic)

This clarification memo (CM) does not result in a change in the protocol informed consent document. The Division of AIDS (DAIDS) has determined that these protocol changes and clarifications should be implemented immediately in response to the SARS-CoV-2 pandemic, which poses a safety risk to participants and site staff. Sites do not need institutional review board (IRB) approval prior to implementing this CM.

DAIDS does not require sites to forward this CM to their IRB; however, sites must follow their IRB's policies and procedures. If IRB review of CMs is required at a site, the site must submit this document for review.

Each site should file a copy of this CM with the protocol for reference.

The team is implementing this CM to provide guidance to sites about the final study visit completion under Final Version 1.0, the Step 2 registration under Final Version 2.0, alternate methods to conducting the study visits, including data collection and documentation, and continuation of ART regimen for the entire duration of the study.

The following are clarifications (noted in bold) to Protocol A5354, Version 2.0, 12/31/19, titled "Effect of Antiretroviral Treatment Initiated During Acute HIV-1 Infection on Measures of HIV-1 Persistence and on HIV-1-Specific Immune Responses." These clarifications will be included in the next version of the A5354 protocol if it is amended at a future date.

1. Only participants who are currently in follow-up and have completed the final study visit (week 72) under Final Version 1.0 AND who provide consent on their willingness to participate in Final Version 2.0 will be permitted to register to Step 2 of the study. NOTE: Participants who have already been discontinued from the study are not permitted to re-enroll at this time.

   If a participant is unable to come to the clinic for the Step 2 registration visit, sites are encouraged to perform an alternative consenting process (e.g., telephone, telehealth) provided sites inform their IRB about this alternate consent method.

2. Section 5.1, Regimens, Administration, and Duration, was updated to include the following clarifications:

   Study treatment is defined as STR elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (EVG/COBI/FTC/TAF) or bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF), which will be provided through the study.

   NOTE**S**: Switching of ARV regimen is allowed (i.e., from EVG/COBI/FTC/TAF to BIC/FTC/TAF, or vice versa, or to an alternate non-study-provided ART) without consulting the CMC. **Sites should ensure that participants who are enrolled into Step 2 have an adequate supply of study-provided drug (i.e., up to a 3-month provision) until a participant is able to come to the clinic for a study visit. If a participant is not able to return to the clinic for any reason and needs a refill of study-provided drug, sites must contact their protocol team pharmacist (via the Clinical Management Committee (CMC) at actg.cmcA5354@fstrf.org) to establish the procedures for shipping study-provided drug to the participant.**

3. Section 6.3.3, Post-Entry Evaluations, was updated to include the following clarifications at the end of the section:

   Remote Data Collection
   Study visits may be conducted remotely (e.g., telephone, telehealth) in the following situations:
   - **A participant is unable to attend a visit because of personal illness, illness among others in his or her home, or local conditions or guidelines restricting travel to the clinic.**
   - **The site is temporarily unable to conduct non-essential visits in the clinic; the site must inform the CMC when it has to stop non-essential visits.**

   Regardless of the situation, sites should document which visits were conducted remotely, attempt to obtain as much of the visit-specific required information, based on the SOE, as possible, and record it on the relevant eCRF. The impacted visits and rationale must be reported and documented, following instructions provided by the team or network leadership.

Implementation of remote data collection is expected to be time-limited in relation to the SARS-CoV-2 pandemic. In consultation with ACTG Network leadership and the study sponsor, the protocol team will determine when remote data collection is no longer permitted. When such a determination is made, study sites will be formally notified and instructed to inform their IRBs/ECs as needed.

A5354

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

A Limited-Center Trial of the AIDS Clinical Trials Group (ACTG)

Sponsored by:

The National Institute of Allergy and Infectious Diseases

Industry Support Provided by:
Gilead Sciences, Inc.

Non-IND Protocol

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DAIDS Clinical Representative:	**Lawrence Fox, MD, PhD**
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FINAL Version 2.0
December 31, 2019

A5354
FINAL Version 2.0
12/31/19

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

SIGNATURE PAGE

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable US Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

Principal Investigator: _____
Print/Type

Signed: _____ Date: _____
Name/Title

TABLE OF CONTENTS

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A5354 will be implemented at US and non-US clinical research sites (CRSs) with active screening programs for acute HIV-1 infection (AHI).

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STUDY MANAGEMENT

Most questions concerning this protocol should be sent to actg.corea5354@fstrf.org via e-mail. The appropriate team member will respond with a "cc" to the appropriate team e-mail group. A response should generally be received within 24 hours (Monday-Friday).

Protocol E-mail Group
Sites should contact the Computer Support Group at the Data Management Center (DMC) as soon as possible to have the relevant personnel at the site added to the actg.protA5354 e-mail group. Include the protocol number in the e-mail subject line.

- Send an e-mail message to actg.user.support@fstrf.org

Clinical Management
For questions concerning entry criteria, Fiebig staging, primary endpoint determination outcome, alternative study medication use (e.g., locally available regimens), clinical management, concomitant medications, and coenrollment, contact the study's CMC, which is a subgroup of the A5354 protocol team.

- Send an e-mail message to actg.cmcA5354@fstrf.org. Include the protocol number, patient identification number (PID), and a brief relevant history.

Laboratory
For questions specifically related to laboratory tests, contact the protocol Immunologist and Virologists.

- Send an e-mail message to actg.cmcA5354@fstrf.org (AT**TENTION**: Scott Sieg [Immunologist] and John Mellors and Gert van Zyl [Co-Virologists]).

Data Management

- For nonclinical questions about transfers, inclusion/exclusion criteria, electronic case report forms (eCRFs), registration, and other data management issues, contact the Data Manager. Electronic CRFs (eCRFs) completion guidelines and participant completed CRFs can be downloaded from the FSTRF website at www.fstrf.org.
- For transfers, reference the Patient Transfer from Site to Site SOP 119, and contact **Tydie Higgins at thiggins@fstrf.org directly**.
- For other questions, send an e-mail message to **actg.teamA5354@fstrf.org (ATTENTION: Tydie Higgins)**. Sites may use their discretion to determine whether the CMC should be copied on these messages.
- Include the protocol number, PID, and a detailed question.

Participant Registration
For participant registration questions or problems and study identification number SID lists.

- Send an e-mail message to rando.support@fstrf.org or call the Statistical and Data Analysis Center (SDAC)/DMC Randomization Desk at 716-834-0900 x7301.

Computer and Screen Problems
Contact the SDAC/DMC programmers.
- Send an e-mail message to actg.**user**support@fstrf.org or call 716-834-0900 x7302.

Protocol Document Questions
For questions concerning the protocol document, contact the Clinical Trials Specialist. Send an e-mail message to actg.corea5354@fstrf.org (AT**TENTION**: Jennifer Tiu).

Copies of the Protocol
To request a hard copy of the protocol, send a message to ACTGNCC@s-3.com via e-mail. Electronic copies can be downloaded from the ACTG website **at** https://www.actgnetwork.org.

Product Package Inserts and/or Investigator Brochures
To request copies of product package inserts or investigator brochures, contact the DAIDS Regulatory Support Center (RSC) at RIC@tech-res.com or call 301-897-1708.

Protocol Registration
For protocol registration questions, send an e-mail message to Protocol@tech-res.com or call 301-897-1707.

Protocol Activation
For questions related to protocol activation at US sites, contact the Clinical Trials Specialist.
- **Send an e-mail message to actg.teamA5354@fstrf.org (ATTN: Jennifer Tiu).**

For questions related to protocol activation at non-US sites, contact the ACTG Site Coordination Group.
- **Send an email message to actgsitecoordination@s-3.com**

Study Product
For questions or problems regarding study product, dose, supplies, records, and returns, contact Oladapo Alli, Protocol Pharmacist, at oladapo.alli@nih.gov.

Study Drug Orders
Call the Clinical Research Products Management Center (CRPMC) at 301-294-0741.

Expedited Adverse Event (EAE) Reporting/Questions
Contact DAIDS through the RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com or call 1-800-537-9979 or 301-897-1709; or fax 1-800-275-7619 or 301-897-1710.

Telephone Calls
Sites are responsible for documenting any phone calls made to A5354 team members.
- Send an e-mail to actg.corea5354@fstrf.org.

Protocol-Specific Web Page

Additional information about management of the protocol can be found on the protocol-specific web page (PSWP).

GLOSSARY OF PROTOCOL-SPECIFIC TERMS

Ab	antibody
AE	adverse event
Ag	antigen
AHI	acute HIV-1 infection
ALT	alanine aminotransferase
ART	antiretroviral therapy/treatment
AST	aspartate aminotransferase
ARV	antiretroviral (or antiretroviral drugs)
β-HCG	β-human chorionic gonadotropin
BIC/FTC/TAF	**bictegravir/emtricitabine/tenofovir alafenamide**
CAHD	cell-associated HIV-1 DNA
CDC	Centers for Disease Control and Prevention
CMC	Clinical Management Committee
CMIA	chemiluminescence immunoassay
CNS	central nervous system
CrCl	creatinine clearance
CSF	cerebrospinal fluid
DHHS	US Department of Health and Human Services
EC	Ethics Committee
EIA	enzyme immunoassay
EVG/COBI/FTC/TAF	elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide
FDA	Food and Drug Administration
HLA	human leukocyte antigen
IRB	Institutional Review Board
LTR	long terminal repeat
PBMC	peripheral blood mononuclear cells
PR	protease
qPCR	quantitative PCR
RE	regulatory entity
RT	reverse transcriptase
SANBS	South African National Blood Service
S/CO	signal-to-cutoff ratio
SMC	Study Monitoring Committee
STR	single tablet regimen
VF	virologic failure
WB	Western blot

SCHEMA

A5354
Effect of Antiretroviral Treatment Initiated During Acute HIV-1 Infection on Measures of HIV-1 Persistence and on HIV-1-Specific Immune Responses

DESIGN

This is a phase II, prospective, open-label **two-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 endpoint 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 **the optional** cerebrospinal fluid collection via lumbar puncture **(LP)** and gut biopsy via flexible sigmoidoscopy. **A target of 25 participants are estimated to have each of the above mentioned optional procedure.** Participants may **consent** to one or **both** 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 the criteria for study discontinuation (refer to 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 under protocol Version 1.0 will be allowed to re-enroll in protocol Version 2.0 if, in the interim, these participants meet Step 2 eligibility criteria. The "re-enrolled" participants will be placed in the Fiebig group in which they were initially enrolled in 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) ***(This study group is closed to enrollment under protocol Version 1.0.)***

Group 3: Fiebig V (reactive HIV-1 antibody and positive Western blot or Geenius HIV-1/HIV-2 without p31 band) ***(This study group is closed to enrollment under protocol Version 1.0.)***

Participants who meet criteria for enrollment and are found to be in Group 2 or 3 based upon Fiebig staging at time of ART initiation will be followed in those groups per protocol. 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 and HIV negative participants will be replaced.**

The Clinical Management Committee of the A5354 protocol team will review the Fiebig staging.

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

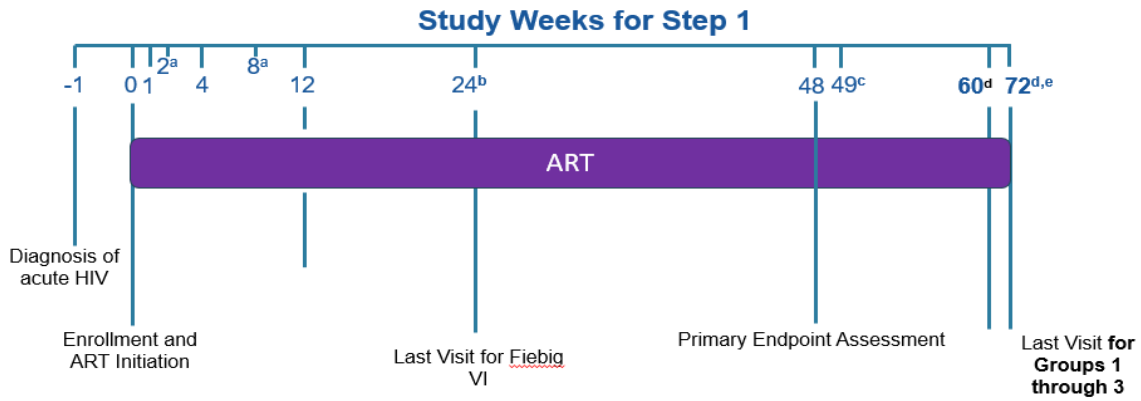
SAMPLE SIZE — A total of 196 participants. Each study group had a target enrollment of 50 participants under protocol Version 1.0. From these study groups, up to 25 eligible participants will be asked to consent to each optional procedure.

POPULATION — Men and women 18 years and older with AHI.

NOTE: Pregnant and breastfeeding women may enroll in the study.

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.

Schema Figure 1: **Study Weeks for Step 1**



[a]Evaluations at weeks 2 and 8 will be performed via telephone.

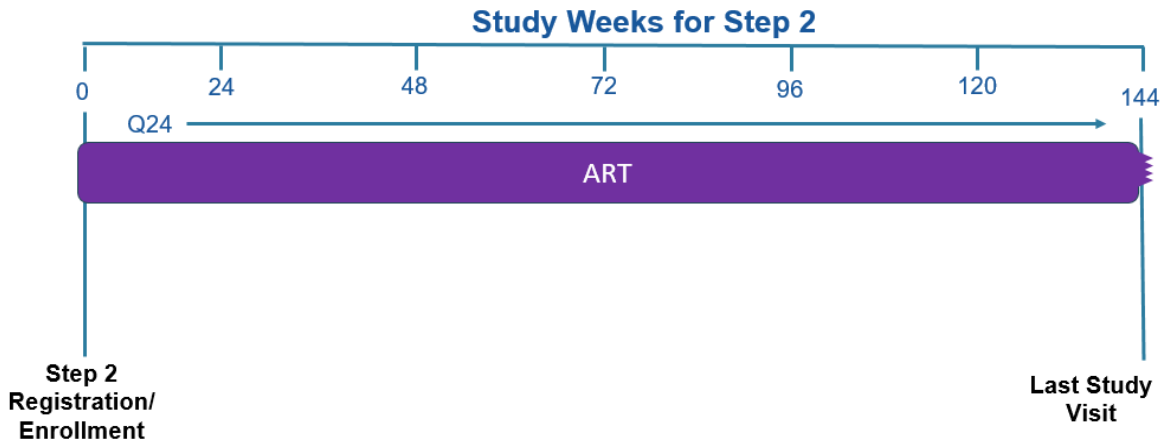
[b]**Week 24 is the last study visit on Step 1 for confirmed Fiebig VI.**

[c]**Optional week 49 visit if insufficient blood drawn at week 48 for the primary endpoint.**

[d]**Optional procedures and large volume blood collection may be performed between weeks 60 and 72.**

[e]**Week 72 is the last study visit on Step 1 for Groups 1, 2, and 3 participants.**

Schema Figure 2: **Study Weeks for Step 2**



1.0 HYPOTHESIS AND STUDY OBJECTIVES

1.1 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**.

1.1.1 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**.

1.1.2 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**.

1.2 Primary Objective

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.

1.3 Secondary Objectives

1.3.1 To evaluate HIV-1-specific CD4+ and CD8+ T-cells by flow cytometry prior to ART initiation and while HIV-1 RNA is suppressed on ART:
   a. magnitude and distribution of CD4+ and CD8+ T-cells responses to nef/tat/rev/vpr/vpu, gag, pol and env
   b. quality of T-cell responses (i.e., frequency and type of polyfunctional responses)

1.3.2 To assess the amount of unspliced HIV-1 RNA in 5 million blood-derived CD4+ T-cells prior to ART initiation and while HIV-1 RNA is suppressed on ART.

1.3.3 To assess cell-associated HIV-1 RNA to DNA ratio in participants with quantifiable HIV-1 DNA prior to ART initiation and while HIV-1 RNA is suppressed on ART.

1.3.4 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.

1.4 Exploratory Objectives

1.4.1 To assess the frequencies of non-transmitted epitope escape mutants prior to ART initiation and after 48 weeks of ART **on Step 1 by sequencing HIV variants, including single genome sequencing and next generation (deep) sequencing**.

1.4.2 To investigate CAHD in CD4+ T-cells and across subsets (e.g., naïve, central memory, effector memory), single copy HIV-1 RNA, and HIV-1-specific immune responses in blood; CAHD in gut; and CAHD and single copy HIV-1 RNA in cerebrospinal fluid (CSF) at any time **on Step 1** from week 60 to week 72 in participants who **consent and** undergo the optional procedures and have available data.

1.4.3 To characterize the effects of early ART on innate immune responses during and after AHI, including natural killer cell subset frequencies, phenotype, and function prior to ART initiation and while HIV-1 RNA is suppressed on ART.

1.4.4 In participants with detectable CAHD at any time point prior to ART initiation and while HIV-1 RNA is suppressed on ART, to quantify episomal HIV-1 DNA by qPCR for 2-LTR circles and derive the quantity of integrated HIV-1 DNA from the total and unintegrated DNA (unintegrated linear DNA or 1-LTR circles would have a negligible contribution after 48 weeks of therapy **on Step 1**).

1.4.5 To investigate the relationship between genetic factors (human leukocyte antigen [HLA] typing and CCR5 delta-32 genotyping) and virologic and immunologic outcomes prior to ART initiation and while HIV-1 RNA is suppressed on ART.

1.4.6 To investigate integration sites prior to ART initiation and while HIV-1 RNA is suppressed on ART.

1.4.7 To store samples for future evaluations of HIV-1 reservoirs with novel assays, HIV-specific immune responses, viral sequencing, and genetic factors.

1.4.8 To characterize the immunologic, virologic, and clinical characteristics of AHI, including response to ART.

1.4.9 To evaluate the performance of HIV diagnostic assays during AHI, including testing algorithms to distinguish between acute infection, chronic infection, and non-infection.

2.0 INTRODUCTION

2.1 Background

Persistent cellular reservoirs of HIV-1 are established very early in AHI [1]. Most proviral DNA is in CD4+ T-cells, particularly central and transitional memory T-cells that persist through homeostatic, antigen-driven, or other mechanisms of proliferation [2]. Very early ART may limit the seeding of cellular reservoirs of HIV-1 [3-5, **6**]. Theoretically, ART initiated in the earliest Fiebig stages could limit the size and genetic diversity of the HIV-1 reservoirs, thereby improving the chance of an HIV-1 cure. Paradoxically, however, post-treatment controllers in the VISCONTI cohort were mainly those treated in later Fiebig stages, i.e., IV-V [**7**]. There are limited data assessing the impact of the timing of ART initiation during AHI on sensitive measures of reservoir size and on virologic and immunologic markers of immune control of HIV-1. Several large studies of early ART in acute and recent HIV-1 infection such as SPARTAC were designed to evaluate clinical outcome and CD4+ T-cells and HIV-1 RNA set point after ART interruption [**8**]. The RV254 Thai study has enrolled mainly Fiebig I to III AHI participants, which limits its relevance to later Fiebig stages that are more commonly diagnosed in routine patient care. One of the largest studies evaluating residual viremia, proviral DNA and infectious virus after early ART had only 27 participants [4]. Although CHAVI has provided detailed descriptions of the natural history of immunologic events in acute and early HIV-1 infection, their studies did not focus on persons treated with modern ART. A thorough study is thus needed to define the impact of early ART on virologic and immunologic outcomes that are relevant to achieving HIV-1 cure [**9**]. Current Fiebig staging utilizes HIV-1 RNA, p24 antigen, 3rd generation enzyme immunoassay (EIA, IgM-sensitive), 2nd generation EIA (IgG-sensitive) and Western blot (WB) to categorize AHI into five stages [**10**].

The duration of these stages varies across individuals and is dependent on the rapidity of the HIV-1-specific antibody response. The protocol team hypothesizes that timing of ART initiation during different stages of AHI will be predictive of the magnitude and characteristics of HIV-1 reservoirs and the quantity and quality of HIV-1-specific immune responses. Longitudinal analysis is needed to evaluate the ability of this staging system to discern the effects of ART instituted in each study group on HIV-1-specific immune responses and HIV reservoirs.

<u>Virologic Outcomes of Interest after Early ART</u>
As noted above, the impact of early ART on the persistence of HIV-1-infected cells has been limited by the number of individuals studied and the detection limits of the assays used to detect persistent HIV-1. Assay detection limits can be improved by sampling larger numbers of cells for HIV-1 nucleic acid. Accordingly, large numbers of CD4+ T-cells (5 million) will be assayed for HIV-1 DNA as the primary study endpoint [**11**]. Having no HIV-1 DNA in CD4+ T-cells detected by sensitive methods following ART is likely an indication of a limited HIV-1 reservoir that may improve the chance of continued virologic control after ART withdrawal. The timing of ART initiation could affect the number and location of HIV-1 integration sites, which in turn could affect clonal expansion, proviral expression, and responsiveness to anti-latency treatments **[12-14**].

The impact of ART timing on HIV-1 integration sites could be evaluated utilizing samples collected during this study.

Immunologic Outcomes of Interest after ART

The earliest HIV-1-specific T-cell responses are predominantly oligofunctional with multifunctional response increasing with time after infection. The epitopes recognized by T-cells during AHI may be narrow in range and non-immunodominant. Escape mutations can occur early after infection in some individuals and may impact virologic control [**15**]. Early ART, depending on the time of its initiation, may prevent the generation of multifunctional T-cell responses [**16**], but may also preserve HIV-1-specific CD4+ and CD8+ T-cell responses [**17-22**]. These observations were mainly derived from small studies of patients whose stage of AHI was not well characterized. In addition, the impact of the timing of ART initiation on the frequency of viral immune escape variants is undefined, particularly for minor escape variants that can only be detected by single genome sequencing or next generation sequencing methods. Immune escape variants pose a significant obstacle to immune control of HIV-1; thus identifying the optimal timing of ART initiation to prevent their emergence is an exploratory objective of this study.

To achieve sustained virologic control of HIV-1 (ART-free HIV-1 remission) and to clear virus expressing cells, it is likely that immune-based therapies will be needed in addition to latency-reversing agents, and responses to such therapies may differ depending on the pre-existing immune responses to HIV-1. Thus, the optimal strategy for manipulating the immune system may be different in infected individuals who initiate ART at different Fiebig stages. Defining if and how immune responses persist or evolve over time in infected individuals treated during AHI, and how they relate to reservoir size and viral diversity, will increase our understanding of critical host-viral interactions and inform the development of immune-based therapies for HIV-1 cure.

Tissue HIV-1 Reservoirs

HIV-1 preferentially persists in the gut mucosa, therefore, larger reservoir sizes in gut tissue compared with the peripheral blood are observed even after successful ART [23, **24**]. While early ART is more beneficial than later ART in reducing HIV-1 DNA in gut, there are limited data on ART effects on the types of latently infected cells [2**5**-2**8**]. In the central nervous system (CNS), CNS inflammation and CSF viremia are reported within days following HIV-1 exposure [2**9**]. Latently infected cells and inflammation persist in the brain after suppressive ART, but it is not well understood how early ART impacts infected cells, low level virus, inflammation, and neuronal injury in the CSF. Failure to mount an adequate immune response to control viral replication and eliminate HIV-1-infected cells in these compartments could directly jeopardize HIV-1 cure.

The Contribution of A5354 to the Understanding of the Effects of Early ART on HIV-1 Persistence and Immune Responses

The investigators of this study have engaged investigators of other NIH-funded early treatment cohort studies and received enthusiastic response for further cross-study collaborations. All groups aim to identify acutely infected individuals and characterize their HIV-1 reservoir size after treatment. There are important differences between the studies as detailed in Table **2.1-1**. Notably, A5354 is unique in its broad inclusion of

individuals across continents who display different genetic profiles and HIV-1 clades, its primary endpoints, and its plans for extended follow-up to facilitate enrollment in interventional trials.

The primary endpoint for A5354 is novel. It is the only study that will extensively interrogate the HIV-1 reservoir after ART by performing tests to identify CAHD in large numbers of CD4+ T-cells. It will secondarily evaluate cell-associated HIV-1 RNA and extensively evaluate the HIV-1-specific immune responses to all HIV proteins, providing important information that will inform strategies for future immune-based therapies. The step-wise approach to performing invasive procedures is innovative and cost-effective. Herein, this study will further investigate persistence of HIV-1 in tissues in those participants whose peripheral blood yields no detectable HIV-1 DNA.

The RV254 Thai cohort establishes and characterizes an AHI cohort in a high risk population. This Division of AIDS (DAIDS)/Department of Defense-funded study commenced in 2009 and as of December 2015 has over 300 participants enrolled. The study is continuing as an open enrolling cohort with an unlimited sample size. The study is conducted at one site in Bangkok with all enrollees being Thai. It has shown so far that HIV-1 reservoir size is small when ART is initiated early. The cohort primarily consists of Fiebig III followed by Fiebig I individuals, limiting the evaluation of outcomes across all Fiebig stages.

The South African cohort, “A Prospective Study of the Virology and Immunology of HIV-1 Infection in South African Blood Donors: Implementation of ART in Very Early HIV-1 Infection and Evolution of Elite Controllers” is a National Heart, Lung, and Blood Institute funded protocol that is being developed as a collaboration between the University of California at San Francisco and the South African National Blood Service (SANBS). The acutely infected individuals will be identified within the SANBS catchment areas and referred for treatment at study sites.

Table **2.1-1.** A5354 and Other NIH-funded Early Treatment Cohort Studies

Study	A5354	RV254 Thai Cohort	South African Cohort
Sites	US and international	Single site in Bangkok	5 sites in 4 provinces
Circulating HIV-1 clades	Multiple clades represented	Predominantly CRF01_AE	Predominantly C
Number of participants	**196**	≥**5**00 with unlimited numbers enrolled	75
Primary objectives	Characterize reservoir/HIV-specific immunity after early ART Cohort of HIV cure research candidates	Characterize reservoir/HIV-specific immunity after early ART Cohort of HIV cure research candidates	Determine Fiebig stages and demonstrate linkage to care for acute HIV-1 infected patients Measure the size of the reservoir after early ART
Fiebig stages	**Target 50 for each Fiebig** I/II, **Fiebig** III/IV, **Fiebig** V	Fiebig I to V (~40% F I/II, 50% F III, 10% F IV/V)	75 Fiebig I/II
Identification of acute HIV-1 infected participants	Performed routinely at the study sites and not part of the protocol	Screening performed as part of the protocol using nucleic acid test (NAT) and sequential EIA	Screening performed as part of the protocol using NAT
Time from enrollment to ART initiation	Same day	Mean 1 day (range 0-7)	4 days to 2 weeks
ART regimens	EVG/COBI/FTC/TAF **and BIC/FTC/TAF** with some receiving locally available alternatives	TDF/FTC/EFV with some receiving RAL and MVC intensification	TDF/FTC+RAL (week 0-24) TDF/FTC/EFV (week 26-96)
Virologic assessments	CAHD in 5 million CD4+ T-cells (highest sensitivity) as primary endpoint and cell-associated HIV-1 RNA as secondary endpoint	DNA and inducible RNA in small blood volumes except for participants who undergo leukapheresis	DNA and cell-associated RNA in small blood volumes except for participants who undergo leukapheresis

Study	A5354	RV254 Thai Cohort	South African Cohort
Immunologic assessments	Magnitude, distribution, quality of T-cell responses to nef/tat/rev/vpr/vpu, gag, pol and env	Magnitude and breadth of T-cell responses to gag, pol, nef and env and other cellular responses (B, NK) Markers of immune activation	Clinical CD4+ T-cell measurement
Invasive procedures	One time after ART: gut biopsy via flexible sigmoidoscopy and lumbar puncture in **a subset of participants after 48 weeks of ART on Step 1**	Leukapheresis, gut and lymph node biopsy, lumbar puncture, genital secretion as optional procedures at multiple time points before and after ART	Leukapheresis at 3 time points after ART

Acute HIV-1 Infection Identification

Best effort must be taken to identify and treat persons with AHI. Several strategies may be employed with the goal of being able to enroll participants **across the** study group**s** (Fiebig I/II, III/IV, or V) which include the following: 1) Frequent routine HIV-1 testing of high risk patients; 2) Outreach to testing sites and clinic/providers administering pre-exposure and post-exposure prophylaxis; 3) Performance of HIV-1 testing within one week of possible HIV-1 exposure and immediately with any acute retroviral syndrome-like symptoms; 4) Engagement of sites that have implemented 4th generation enzyme immunoassay (EIA) or nucleic acid testing as part of HIV-1 testing as well as outreach to testing sites that conduct such tests.

Strategy for Rapid Diagnosis of AHI and Fiebig Staging

Where practical, we recommend deploying routine screening with 4th generation Ag/Ab combo assays (Abbott ARCHITECT HIV-1/2 Combo or Bio-Rad HIV-1/2 Combo or Siemens AVIDA Centaur HIV-1/2 Combo) with preference for the Abbott ARCHITECT chemiluminescence immunoassay (CMIA) because of the rapid test turn-around-time of approximately 30 minutes versus 2 hours for the Bio-Rad; in addition, the broad dynamic CMIA range has the potential to use the S/CO to establish HIV-1 infection recency **[30]**. Using an algorithm of a 4th generation Ag/Ab assay in combination with emerging technologies for rapid confirmatory and discriminatory HIV-1/2 antibody screening (HIV IgG Multispot or the Geenius assay, once available) [3**1**] followed by rapid HIV-1 RNA testing using either the laboratory-in-a-tube (IQuum/Roche) 30-minute quantitative HIV-1 RNA test or the 60-minute Alere Diagnostics HIV-1/2 RNA assay, it is possible to identify participants in Fiebig I/II on the same day as enrollment.

There are currently four Food and Drug Administration (FDA)-approved 4th generation HIV-1/2 Ag/Ab screening assay platforms that allow for the diagnosis of acute or established HIV-1 infection. All four platforms are able to detect HIV-1 antigen and HIV-1/2 antibodies in blood serum or plasma. One of these platforms is an EIA (Bio-Rad HIV-1/2 Combo Ag/Ab EIA); two are a magnetic microparticle-based CMIA (Abbott ARCHITECT HIV-1/2 Ag/Ab Combo and Siemens ADVIA Centaur HIV-1/2 Ag/Ab Combo assay); and one is a luminex flow-based assay that differentiates HIV-1 antibody from HIV-1 p24 antigen and HIV-1 from HIV-2 antibodies (Bio-Rad BioPlex 2200 HIV Ag-Ab assay). In addition, the recent FDA approval of the confirmatory Bio-Rad Geenius HIV-1/2 lateral flow antibody assay offers an opportunity to replace both the rapid Multispot HIV-1/2 and the WB with a discriminatory rapid (<30 minutes) antibody confirmation of a reactive 4th generation screening test and, in addition, estimates the Fiebig stage based on the detection of HIV-1 gag (p24), pol (p31), and env (gp160, gp41) antibodies and for HIV-2, two env-specific peptides (gp36 and gp140). The available FDA-approved HIV-1 antibody screening and orthogonal confirmatory assays are listed in the A5354 Manual of Procedures (MOPS).

The Centers for Disease Control and Prevention (CDC) 4th generation screening algorithm may be used to identify acute, early, and established HIV-1 infection (see A5354 MOPS). Where available the Geenius assay will be done immediately on site to detect or confirm HIV-1 antibody and to allow a preliminary Fiebig staging. Where

appropriate (e.g., reactive screen and negative antibody assay (e.g., Multispot or Geenius), an HIV-1 RNA will be performed at the site.

An analysis of 39,721 specimens tested from a combined research and clinical testing service showed that the ARCHITECT S/CO clearly distinguished the false positive (0.2%) from AHI (0.1%) and established infection (1.33%) (Figure 2.1-1). Interestingly, 0.7% of the specimens had a S/CO **of** between 0.50 and 0.99. Three (10%) of the 30 AHI had a S/CO of between 0.50 and 0.99, which suggests that in a high-seroincidence population, a S/CO less than 1.0 may identify additional AHI [30, **31**].

We posit that the strength of the S/CO will equate with the likelihood of a particular infection stage in this high seroincidence study population.

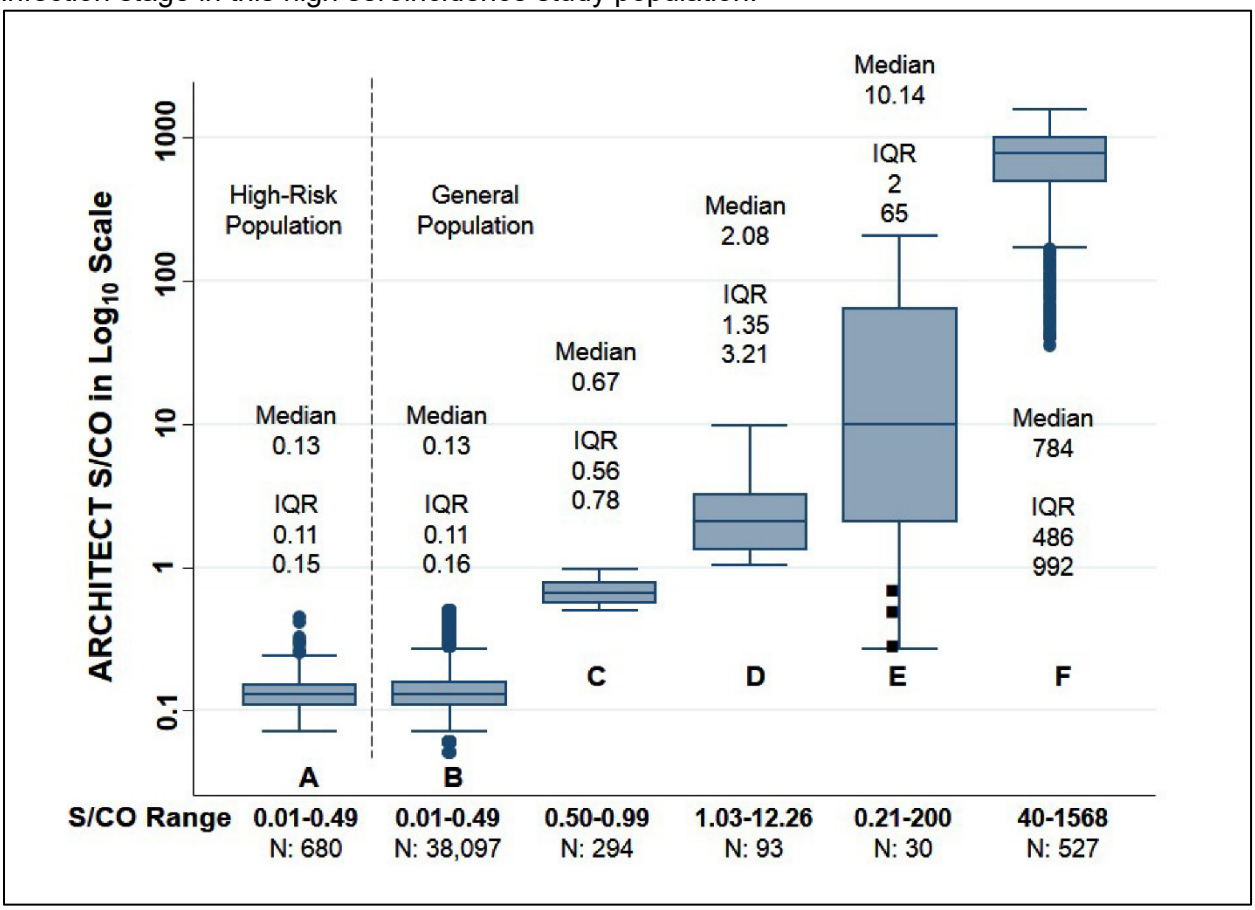


Figure**2.1-**1. Distribution of ARCHITECT S/CO values: A, B: non-reactive; C: intermediately reactive; D: false-positive; E: acute HIV-1 infection; F: established infection [3**2**]

We have shown that the 4th generation ARCHITECT HIV-1/2 Ag/Ab Combo assay in comparison to the 4th generation Bio-Rad Combo provides a greater dynamic S/CO range for determining recent infection (Figure 2**.1-2**). The ARCHITECT S/CO was also shown to be useful for differentiating acute from recent and established HIV-1 infections

based on an indeterminate or confirmed WB with or without the presence of HIV-1 p31 antibody (Figure **2.1-**3). However, in conjunciton with the Geenius assay, the Fiebig classification can be estimated as I-II (RNA or p24 antigen positive [or both] and no antibody) versus III-IV (IgM/IgG with negative or indeterminate WB) versus V (confirmed WB and no anti-p31) versus VI (confirmed WB and anti-p31).

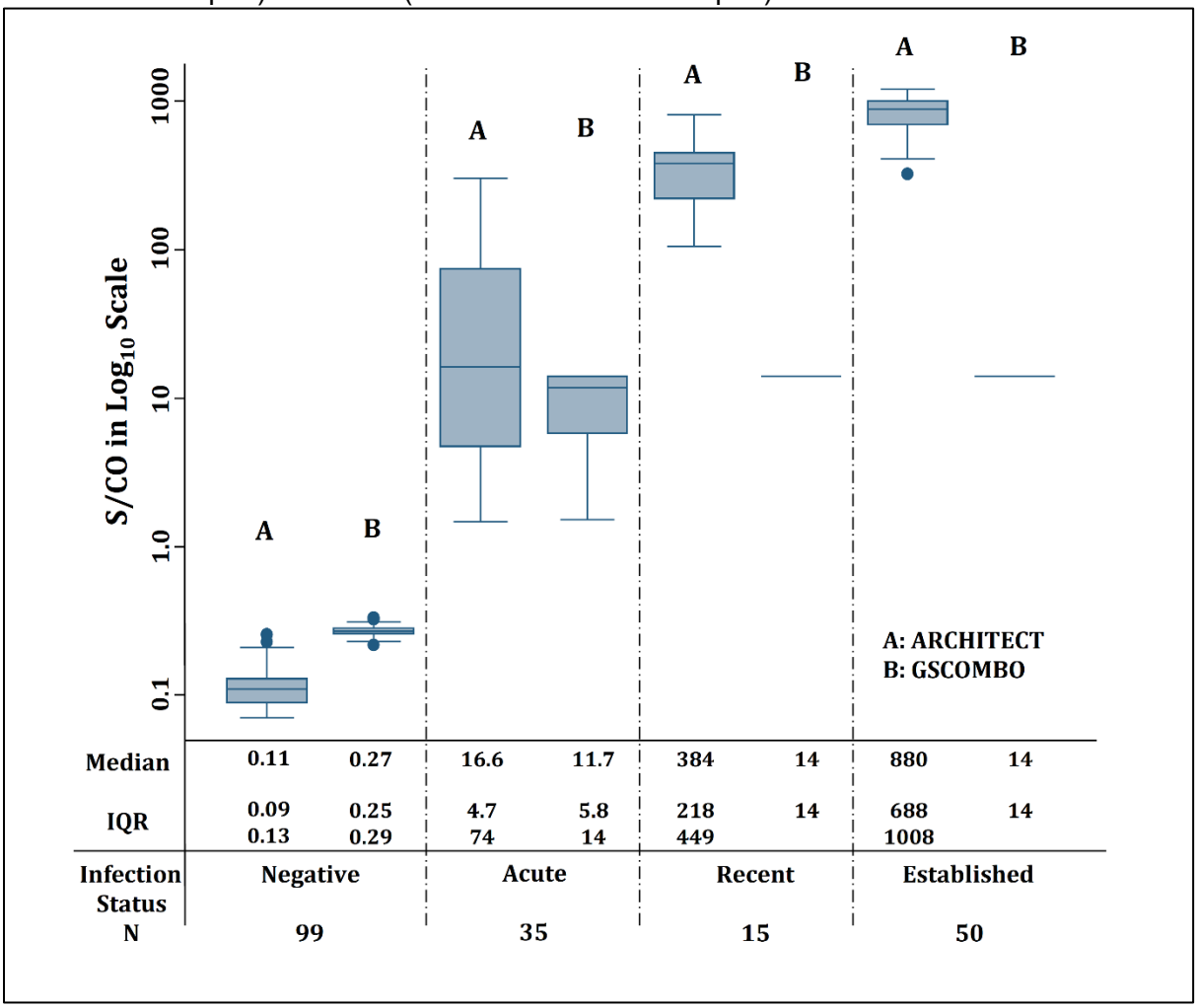


Figure 2**.1-2**. ARCHITECT versus GSCOMBO for S/CO values over the range of HIV-1 infection [3**2**]

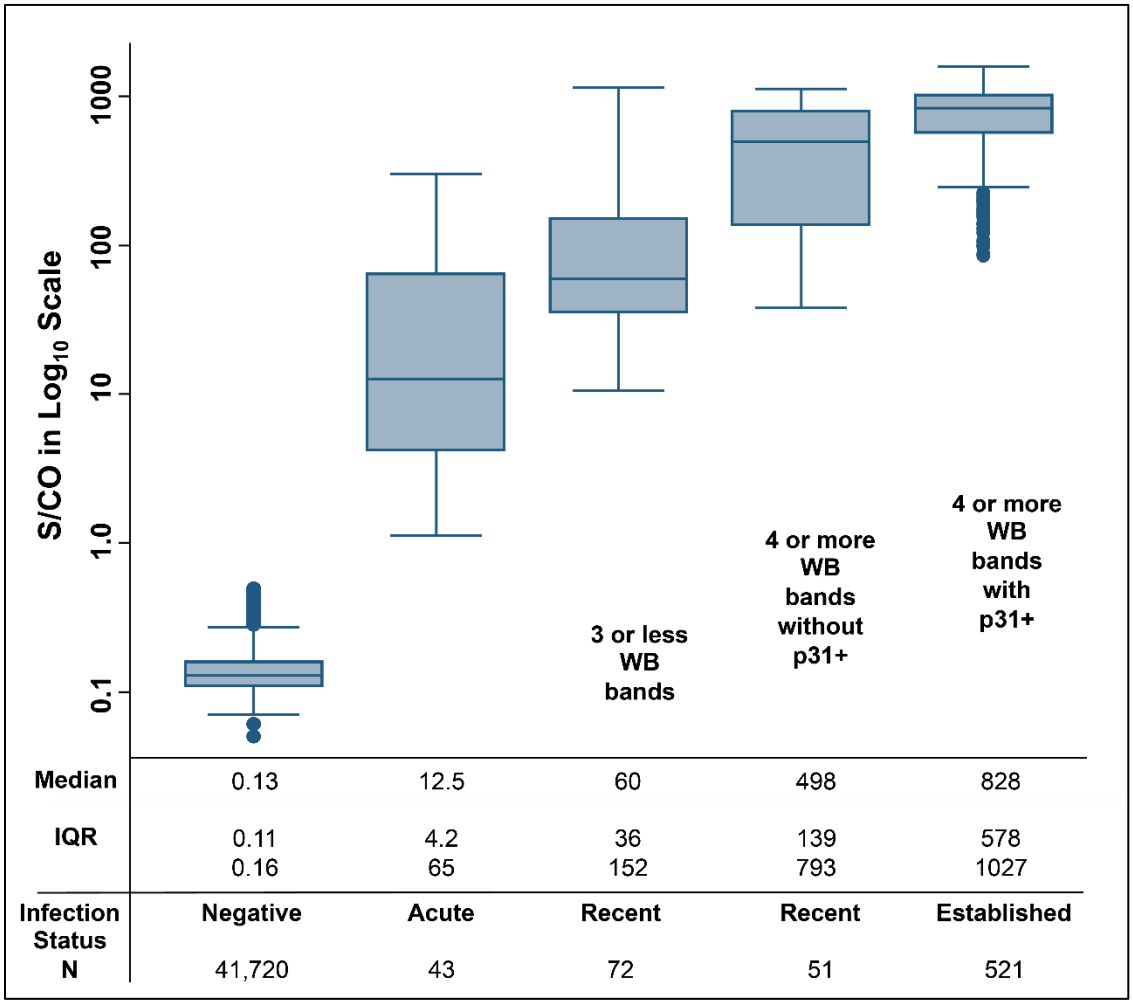


Figure **2.1-**3. ARCHITECT S/CO for HIV-1 infection recency [3**2**]

For the purpose of this study, a S/CO ratio ≥10 is considered "positive" in the ARCHITECT assay. However, a S/CO of between 0.5 and 10 that corresponds to HIV-1 p24 antigen positive AHI represents between 4-5.5 $\log_{10}$ HIV-1 RNA copies/mL [**30**]. Therefore, HIV-1 p24 antigen detection at a S/CO of 0.5 may correspond to approximately 6,000 HIV-1 RNA copies/mL (Figure **2.1-**4 below), which is well above the lower limit of assay detection for commercial assays. Participants with a previous ARCHITECT S/CO ratio of <0.5 who now have a value ≥1.0 are eligible to enroll in the study pending the HIV-1 RNA determination. Potential participants with S/CO >0.5 but <10 will require a positive HIV-1 RNA results prior to enrollment (this criterion will decrease the incidence of false-positive HIV-1 infection).

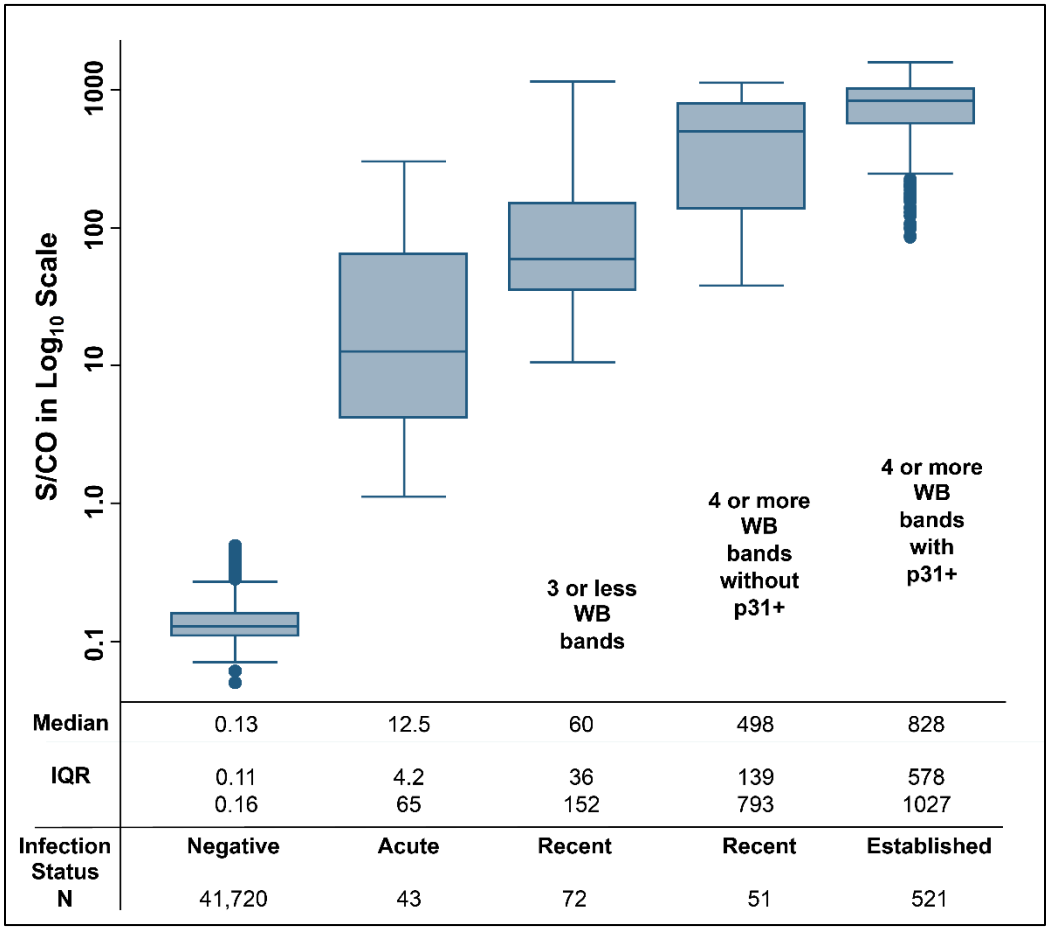


Figure **2.1-**4. The $\log_{10}$ linear relationship between the HIV-1 RNA copies/mL of plasma (abscissa) and the ARCHITECT assay S/CO values (ordinate)

Challenges

The majority (19/23, 83%) of the ACTG international laboratories have an Abbott m2000 system available, 10/23 (43%) also have the Roche COBAS TM, and most of the laboratories conduct HIV-1 RNA testing on-site. However, the turn-around-times (TAT) for results is highly variable from <24 hours up to 14 days even for on-site testing. The average TAT is 4.6 days and the median is 3 days. These times are independent of instrument; i.e., if it takes 2 days for results at one laboratory on the Roche COBAS TM, it also takes 2 days for results at the same laboratory using the Abbott m2000 system. Inclusion criteria for this study are designed to identify individuals during AHI while accommodating differences in testing at various study sites. Fiebig staging will be performed using standardized processes at reference laboratories on samples collected on the day of study entry.

PCR Assay for Detection of CAHD and Cell-associated RNA in CD4+ T-cells
A qPCR assay with single-copy sensitivity targeting a highly conserved region of integrase in the HIV-1 *pol* gene [3**3**] will be used to detect HIV-1 DNA or RNA in 5 million total CD4+ T-cells.

Antiretroviral Regimen
The study will provide the open-label EVG/COBI/FTC/TAF **or BIC/FTC/TAF** to all enrolled participants. The availability of **these** single-tablet regimen (STR) integrase strand transfer inhibitor (INSTI)-based regimen**s** will facilitate the rapid initiation of therapy and reduction in viral load for those identified with AHI. Single-tablet regimens may demonstrate improved adherence and participant satisfaction over multi-pill regimens, and INSTI-containing regimens have repeatedly been shown to result in more rapid reduction in viral load when compared with non-INSTI-based therapy [34, **35**]. EVG/COBI/FTC/TAF **and BIC/FTC/TAF** ha**ve** been approved by the US FDA **and both include** the TAF formulation of tenofovir, which appears to have distinct safety advantages **over TDF**.

TAF is a second generation oral prodrug of tenofovir, a nucleotide analog that when phosphorylated inhibits HIV-1 reverse transcriptase. TAF is metabolized to tenofovir intracellularly and can be used at a fraction of the dose of TDF with lower blood levels and higher intracellular levels. [3**6**] There have been many clinical trials that have included TAF as well as EVG/COBI/FTC/TAF, including a phase II study (GS-US-292-0102) that included 58 participants treated with the regimen with 88.4% having HIV-1 RNA <50 copies/mL at 48 weeks by snapshot analysis compared with 87.9% in those given EVG/COBI/FTC/TDF. Results from the pharmacokinetic study showed that 10 mg TAF resulted in lower blood plasma levels of tenofovir than TDF at 300 mg with approximately five-fold higher levels of tenofovir diphosphate in peripheral blood mononuclear cells (PBMC) than with TDF.

Two phase III registrational trials (GS 104 and GS 111) have been completed and published comparing EVG/COBI/FTC/TDF with EVG/COBI/FTC/TAF in a combined study of more than 1700 ART-naïve individuals [3**7**]. It is noteworthy that although the median CrCl of enrolled participants exceeded 100 mL/minute, the studies did allow for enrollment of participants with CrCl ≥50 mL/minute. The proportion with plasma HIV-1 RNA <50 copies/mL through 48 weeks by snapshot analysis was 90% in the TDF- versus 92% in the TAF-containing regimens with adjusted difference of 2.0%, 95% CI -0.7 to 4.7, meeting pre-specified non-inferiority criteria. Furthermore, there was no difference in response in either group based upon baseline plasma HIV-1 RNA (less than or equal to or greater than 100,000 copies/mL) or CD4+ T-cell count (less than or greater than or equal to 200 cells/μL). Protocol-specified virologic failure (VF) was similar and low in both groups as was emergent drug resistance, and the pattern of such resistance was similar between study groups. The mean increase in CD4+ T-cell count was significantly greater at 48 weeks in the TAF versus TDF group at 230 versus 211 cells/μL, respectively (p=0.024). The frequency of any AE was 90% in both groups with only 1% in each group having drug-related Grade 3 or 4 AEs. Common AEs occurring at least in 5% of individuals were also similar between groups with most frequent being diarrhea, nausea, and headache. Special attention was given to the difference in effect

on renal **function** and bone **density** in these studies since these are recognized AEs associated with TDF use. The TAF group had significantly smaller mean serum creatinine increases than those given a TDF-based regimen (0.08 versus 0.12 mg/dL, p<0.001), less proteinuria (median percent change -3.0 versus 20; p<0.001), and significantly smaller decrease in bone mineral density at spine (mean % change -1.3 versus -2.86; p<0.001) and hip (mean % change -0.66 versus -2.95; p<0.001). There was a significantly greater increase in total, LDL and HDL cholesterol as well as triglycerides in those receiving a TAF- rather than a TDF-containing regimen, although no difference was seen in total cholesterol:HDL ratio. The package insert for EVG/COBI/FTC/TAF allows for treatment of participants with CrCl ≥30 mL/**minute GENVOYA [package insert]. Gilead Sciences, Inc., Foster City, CA; March 2016. http://www.gilead.com/~/media/files/pdfs/medicines/hiv/genvoya/ genvoya_pi.pdf. Accessed Mar 30, 2016].** Participants with CrCl <30 mL/minute can be included in the study if they are able to access appropriate alternate regimens according to local guidelines.

BIC is an HIV-1 INSTI. It has shown synergistic effects when combined with other ARVs including TAF and FTC. It has a median half-life of 15.9 to 20.9 hours [38, 39], and is cleared equally through UGT1A1 glucuronidation and CYP3A4 oxidation. BIC has a superior resistance profile compared to some of the other currently approved drugs in the same class. It is potent against a panel of patient-derived HIV-1 isolates with high-level INSTI resistance. A 10-day dose ranging study of BIC monotherapy showed anti-viral effects in both ARV-naïve and ARV-experienced (INSTI-naïve) people [40]. A randomized, double-blind, placebo controlled Phase II study compared BIC 75mg/FTC 200mg/TAF 25 mg (n=65) versus DTG 50mg/FTC 200mg/TAF 25mg (n=33) in ARV-naïve adults. At week 24, 63 of 65 (96.9%) in BIC group versus 31 of 33 (93.9%) in DTG group had HIV RNA < 50 copies/ml. Adverse events were reported in 85% in the BIC group versus 67% in the DTG group. Most common AEs were diarrhea and nausea. One participant in the BIC group discontinued due to urticarial reaction. No SAEs or deaths occurred [41]. A Phase III randomized, controlled, clinical trial of BIC/FTC/TAF versus DTG/FTC/TAF in ARV-naïve adults showed the former was non-inferior to the latter for virological suppression. There was no treatment-emergent resistance. BIC/FTC/TAF was safe and showed less reduction in eGFR compared to DTG/FTC/TAF. There were no discontinuations due to renal side effects, and changes in lipid parameters were similar between arms (NCT02607956). An ongoing Phase III study comparing the STR of BIC/FTC/TAF (n=314) with DTG/ABC/3TC (n=315) in treatment-naïve adults showed similar viral suppression < 50 copies/mL at week 48: 92.4% with BIC/FTC/TAF versus 93% with DTG/ABC/3TC. The discontinuation rates were similar between arms: 6% versus 5%, respectively. Most common side effects in the BIC versus DTG arms were diarrhea (12.7% versus 13%), headache (11.5% versus 13.7%) and nausea (10.2 versus 22.9%) (NCT02607930). There are four ongoing Phase III switch studies, with three evaluating safety and effectiveness of the switch to BIC/FTC/TAF from any suppressive ARV regimen (NCT02603120, NCT02652624, and NCT02603107). The fourth study (NCT03110380) will perform similar evaluations but with a switch to BIC/FTC/TAF from regimens with DTG and either FTC/TDF or FTC/TAF.

Urgency of ART Initiation

Results of the Strategic Timing of Antiretroviral Treatment (START) study highlight the clinical benefits of initiating ART soon after HIV-1 diagnosis, regardless of CD4+ T-cell count [**42**]. Randomization into early and deferred treatment arms was halted in May 2015, after a scheduled interim review of study data by the Data and Safety Monitoring Board. Over an average follow-up of 3 years, the risk of AIDS, other serious illnesses or death was reduced by 57% among those in the early treatment group compared with those in the deferred treatment group. The study authors reported 42 instances of AIDS, serious non-AIDS illness, or death among those enrolled in the study's early treatment group compared with 96 such events in the deferred treatment group. Similar results were seen in the TEMPRANO study; immediate ART initiation reduced the incidence of death or severe HIV-related illness by 44% as compared with delayed initiation of ART after CD4+ T-cell count dropped below 500 cells/mm^3 [**43**]. These results have impacted treatment guidelines to favor universal treatment of HIV infection as soon as it is diagnosed.

There is a particular urgency to initiate ART during the first few weeks of infection, as this may limit the establishment of HIV latency [3-**6**]. Seeding of the latent HIV reservoir begins very early in AHI and the reservoir increases in size as individuals progress through the Fiebig stages [2, 5]. Fiebig I-IV stages are each projected to last an average of only about 3-5 days and some individuals may progress even more rapidly [**10**]. Investigators anticipate that individuals who have a high-risk for incident HIV-1 infection will be referred for possible enrollment in this protocol upon receipt of laboratory results suggesting recent HIV-1 infection. It is critical that these potential participants be screened for study entry and enrolled into the protocol before they advance in their Fiebig staging. Urgent enrollment and immediate initiation of ART is necessary in order to limit establishment of the latent HIV-1 reservoir and, in this study, to ensure that enrollment includes participants who initiated ART during the very earliest Fiebig stages.

For these reasons, consenting individuals who satisfy criteria for enrollment into this study will begin ART on the day of study entry. HIV-1 RNA testing will also be performed at the time of study entry. ART initiation will not be delayed while awaiting results of HIV-1 tests in participants with high likelihood of having AHI based on inclusion criteria. Results of entry HIV-1 testing will generally be made available within 72 hours.
There is a theoretical possibility that participants will be enrolled but are later discovered not to be infected with HIV-1. Enrollment criteria defined by **signal-to-cutoff ratio (**S/CO**)** of 4th generation EIA do not all include confirmatory HIV-1 testing, but have been shown to have a positive predictive value of nearly 100% [Bob Coombs, personal communication]. In rare cases in which individuals are started on ART and later found not to be infected with HIV-1, these individuals will typically receive three or fewer doses of ART while awaiting the results of HIV-1 RNA testing, which will generally be made available within 72 hours of study entry. ART will be stopped as soon as the negative HIV-1 test result is reported. The safety of ART has been well-documented in HIV-1-uninfected individuals who receive ART for both pre-exposure and post-exposure prophylaxis [4**4-49**]. The brief ART exposure that is a potential, but unlikely, outcome of ART initiation upon study entry is expected to be safe and well-tolerated.

Feasibility of A5354 Enrollment

The ACTG site survey (conducted in late 2014) has illustrated marked enthusiasm and feasibility for enrolling this study within DAIDS networks. Of 43 sites that completed the survey, 63% actively screen for AHI, 78% of sites routinely perform 4th generation EIA for HIV diagnostics, and 60% perform NAT on antibody negative samples. In addition, 84% of sites confirmed the capability to identify and initiate ART in acutely infected participants. Capabilities for performing invasive procedures were high with percentages of sites able to perform each procedure as follows: 75% for leukapheresis, 92% for gut biopsy, 83% for lymph node biopsy, 97% for lumbar puncture, and 97% for genital secretion collection. These sites are located in the US and internationally (Thailand, Kenya, South Africa, and Peru). Specifically, there were 18 sites (6 domestic and 12 international) that estimated enrollment of at least 10 acutely infected individuals per year and can also perform at least one invasive procedure.

We intend to enroll individuals at ACTG sites with experience treating and following HIV-1-infected individuals. Sites will be selected based on the following criteria: 1) actively screen for AHI, 2) track record of identifying acutely infected individuals, 3) demonstrate high retention and viral suppression rates on ART in previous studies, and 4) are capable of performing **large volume blood draw or the optional leukapheresis**.

The study team is confident that it will be feasible to enroll **196** participants in a **36**-month period. As **of May 22, 2019, 202 individuals have been screened for enrollment into the study and 168 participants have been enrolled. Group 1 remains open to accrual and is nearing its target enrollment. Groups 2 and 3 have been fully accrued under protocol Version 1.0.**

Inclusion of Pregnant and Breastfeeding Women

The inclusion of pregnant women in this study helps maintain the feasibility of enrolling an adequate number of female participants in order to achieve overall enrollment goals. A new diagnosis of pregnancy indicates recent unprotected sexual activity that, particularly in high-risk populations, may lead to HIV-1 acquisition. During pregnancy, immunological and hormonal changes affect the mucosa of the genital tract and increase the risk of HIV-1 acquisition [**50, 51**]. Previous studies have demonstrated higher rates of HIV-1 seroconversion among pregnant women than among non-pregnant peers [**51-54**]. The ongoing RV217 cohort study that screens HIV-negative participants at high risk for HIV-1 infection twice weekly to capture AHI observed 25 of the first 48 female participants to be pregnant at some point during the study [Merlin Robb, personal communication]. Both the US Department of Health and Human Services (DHHS) adult and adolescent HIV treatment guidelines and the perinatal HIV treatment guidelines recommend the initiation of ART in all pregnant women with acute or recent HIV-1 infection as soon as possible in order to maximally reduce plasma HIV-1 RNA, both to improve maternal health and prevent perinatal transmission of HIV-1 (AI strength of recommendation) [**55, 56**]. Pregnant participants will not be eligible to volunteer for optional invasive procedures. Blood volumes collected from pregnant participants will be carefully monitored and will be adjusted as needed to maintain the safety of the participant. Breastfeeding practice is common in many countries where the study sites are located. The DHHS guidelines highlight the increased risk of perinatal transmission

of HIV-1 during breastfeeding in women with AHI; therefore, this protocol recommends ART initiation in all HIV-1-infected pregnant and breastfeeding women. Because there are limited data on the safety of EVG/COBI/FTC/TAF **and BIC/FTC/TAF** in pregnant and breastfeeding women, this protocol recommends that study sites secure alternative regimens according to local guidelines for these women until they are no longer pregnant or breastfeeding, at which time, they have the options to stay on the same regimen or use the regimen provided by this study.

Extended Follow-up Period for All Study Groups Enrolled into Step 2
Enrollment into the extended follow-up period (Step 2) for all study groups will require that participants have maintained virologic suppression while on study (i.e., no virologic failure per protocol definition) and have not discontinued from the study for other reasons through week 72. As of the May 22, 2019 monitoring report, we estimated that 74% of participants will qualify for the extended Step 2 follow-up period. The team's intention is to have these participants continue on study for an additional 3 years (after the final Step 1 visit at week 72) with the extended follow-up visits occurring every 24 weeks until 144 weeks after entry to Step 2. Step 2 will involve limited evaluations, and there will be no invasive procedures. We expect that safety laboratory testing will be done according to standard of care outside of the protocol and no less than every 6 months.

Inclusion of Re-enrolled Participants from A5354 Version 1.0 into Step 2
Group 1, 2, and 3 participants who completed the study at week 72 and went off study under protocol Version 1.0 will be allowed to re-enroll if they have maintained virologic suppression and meet the Step 2 eligibility criteria. The "re-enrolled" participants will be placed in the Fiebig group in which they were initially enrolled in under protocol Version 1.0 and will be followed per the Step 2 schedule.

The rationale for the extended follow-up period is to expand the number of available participants for future therapeutic and cure studies without the burden of frequent visits and the cost of study-provided laboratory testing.

2.2 Rationale

The study-provided regimen is STR EVG/COBI/FTC/TAF **or 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.

This study aims to understand the extent of HIV-1 reservoir establishment and HIV-1-specific immune responses across stages of AHI before and after suppressive ART in the context of diverse HIV-1 clades. Such insight will be critical in designing therapeutic strategies and understanding the responses observed to future interventions in cure-related studies. The study will also provide a well-characterized and virologically suppressed cohort of individuals who are well suited to test novel curative interventions

as they emerge. The primary rationale for conducting this study is to achieve the following goals:

1. Design a study to leverage existing infrastructure, resources, and expertise across DAIDS networks to determine the effect of ART initiated during different stages of AHI on HIV-1 persistence and HIV-1-specific immune responses.
2. Make ART available through this study to eligible individuals to initiate ART within a very short time of being diagnosed with AHI.
3. Accumulate a cohort of well-characterized participants who initiated ART during different stages of AHI with expected differences in HIV-1 reservoirs and HIV-1-specific immune responses and who will be well-suited for studies of curative interventions.

3.0 STUDY DESIGN

This is a **P**hase II, prospective, open-label **two-step** study to measure the effects of early ART on the establishment of HIV-1 reservoir and HIV-1-specific immunity. Participants will be enrolled if they fulfill the inclusion criteria for AHI diagnosis within 7 days prior to entry and will have an enrollment visit with the immediate initiation of ART. Plasma and serum samples for Fiebig staging will be collected at the time of ART initiation.

Participants will be followed for up to **216** weeks **in Steps 1 and 2**. Evaluations at weeks 2 and 8 **on Step 1** will be performed via telephone. The primary endpoint is CAHD in 5 million blood-derived CD4+ T-cells assayed by qPCR at week 48 **on Step 1**.

A subset of eligible participants **in Groups 1, 2, and 3 who have completed week 48 on Step 1** will be consented for CSF collection via lumbar puncture and**/or** gut biopsy via flexible sigmoidoscopy. **A target of 25 participants are estimated to have each of the aforementioned optional procedure.** These procedures are optional and participants may **consent** to one or **both** of them, which will be performed at any time from week 60 to week 72 **on Step 1**.

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. Samples of plasma and serum will be collected at the time of ART initiation, and the results of the Fiebig stage at ART initiation will be available within 12 weeks **following entry to Step 1**. In this study, the five Fiebig stages of interest will be simplified into three study groups **as described below** (based on HIV-1 antibody diagnostic profile at time of ART initiation).

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 WB or Geenius HIV-1/HIV-2) ***(This study group is closed to enrollment under protocol Version 1.0.)***

Group 3: Fiebig V (reactive HIV-1 antibody and positive WB or Geenius HIV-1/HIV-2 without p31 band) ***(This study group is closed to enrollment under protocol Version 1.0.)***

Each study group had a target enrollment of 50 participants under protocol Version 1.0. Study Groups 2 and 3 reached their target enrollment under protocol Version 1.0; therefore, the original inclusion criteria from protocol Version 1.0 corresponding to these groups are no longer applicable for eligibility into the study. Participants who meet criteria for enrollment and are found to be in Group 2 or 3 based upon Fiebig staging at time of ART initiation will be followed in those groups per protocol. Participants who are enrolled in Groups 1, 2, and 3 will complete Step 1 of the study at week 72, and willing participants who have completed Step 1 follow-up without meeting the criteria for study discontinuation (refer to section 9.2) will be encouraged to continue on Step 2 and complete study evaluations through 144 weeks of Step 2 follow-up. For those participants who completed the week 72 visit under protocol Version 1.0 and meet eligibility criteria for re-enrollment into Step 2, they will remain on Step 2 for up to 144 weeks of follow-up.

Although participants in Fiebig VI (positive WB or Geenius HIV-1/HIV-2 with p31 band) are not specifically targeted for enrollment in this study, it is possible that a small number of participants will be determined to be in Fiebig VI based on analysis of the entry samples and will be followed on the study for no more than 24 weeks on Step 1. In order to provide time to ensure a smooth transition off of the study without compromising the continuity of ART, participants who are determined to be in Fiebig VI **(based on centralized testing)** will be followed **on study for no more than 24 weeks on Step 1** allowing ample time for them to pursue alternative sources for ART. **Confirmed Fiebig VI and HIV negative participants will be replaced.**

NOTE: The CMC will review the Fiebig stage determination outcome and will ensure that these results are communicated to the sites (**refer to** details in A5354 MOPS).

The study-provided regimen **is** STR EVG/COBI/FTC/TAF **or BIC/FTC/TAF**. EVG/COBI/FTC/TAF **and BIC/FTC/TAF are** being provided for the following reasons: 1) rapid decline in viremia and potential to reduce reservoir seeding with INSTI, 2) generally low frequencies of transmitted INSTI resistance, 3) potentially improved adherence with STRs, and 4) the TAF formulation may have fewer renal and bone adverse effect **than TDF**. Other non-study-provided ARV regimens will also be allowed for participants 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. Investigators may choose an appropriate non-study-provided ARV regimen for **any of these reasons**. Alternative regimens will not be provided by the study, but their use does not preclude participation in the study. Th**is** flexibility is expected to enhance enrollment and adherence to the study requirements. Use of alternative regimens other than EVG/COBI/FTC/TAF **or BIC/FTC/TAF** must not delay the initiation of ART.

4.0 SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 **Step 1** Inclusion Criteria **for Group 1**

4.1.1 Appropriate documentation from medical records of diagnosis of AHI within 7 days prior to enrollment, that includes one of the following:

a) A detectable HIV-1 RNA within 28 days prior to study entry AND a non-reactive HIV-1 antibody within 7 days prior to entry

OR

b) ARCHITECT or GSCOMBO S/CO ≥10 within 7 days prior to entry AND a non-reactive HIV-1 antibody within 7 days prior to entry

OR

c) ARCHITECT or GSCOMBO S/CO ≥1 within 7 days prior to entry AND a non-reactive HIV-1 antibody within 7 days prior to entry AND a known prior S/CO <0.5 within 90 days prior to entry

OR

d) ARCHITECT or GSCOMBO S/CO >0.5 but <10 within 7 days prior to entry AND a non-reactive HIV-1 antibody within 7 days prior to entry AND detectable HIV-1 RNA within 7 days prior to entry

NOTE A: HIV-1 RNA result must be reported from an FDA-approved **or CE-marked** assay.

NOTE B: **Refer to** section 10.5 for additional information on estimated Fiebig group according to each inclusion criterion above. The protocol team will notify the sites if some criteria may no longer be used because accrual is completed in certain Fiebig groups.

NOTE C: Specimens for the testing specified in section 4.1.1 above may be collected on the day of study entry provided the testing result is available prior to enrollment.

4.1.2 Men and women age ≥18 years.

4.1.3 Ability and willingness of candidate to provide written informed consent.

4.1.4 Ability and willingness to initiate ART at enrollment.

4.1.5 Ability and willingness to participate in scheduled study visits **per the schedule of events (SOE)**.

4.1.6 Female candidates of reproductive potential who are not pregnant at the time of enrollment **and who** will receive the study-provided EVG/COBI/FTC/TAF **or BIC/FTC/TAF** must agree not to participate in the conception process (i.e., active attempt to become pregnant, in vitro fertilization), and if participating in sexual activity that could lead to pregnancy, the female candidate must agree to use at least one reliable form of contraceptive while receiving study-provided treatment.

Female candidates are considered to be of reproductive potential if **all** of the following conditions apply:

- Candidate has experienced menarche.
- Candidate has not undergone bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.
- Candidate has not experienced menopause, defined as lack of menstruation within the preceding 12 months.

Acceptable contraceptive methods include:

- Condoms (male or female) with or without a spermicidal agent
- Diaphragm or cervical cap with spermicide
- Intrauterine device
- Hormonal contraceptive

Female candidates who are not of reproductive potential or whose male partner(s) has documented azoospermia are not required to use contraceptives. Any statement of self-reported sterility or that of her partner must be entered in the source documents.

NOTE: Acceptable documentation of lack of reproductive potential is oral or written documentation from the individual.

4.1.7 Pregnant and breastfeeding women who agree to initiate a non-study-provided ARV regimen per section 8.9, and who otherwise meet the eligibility requirements.

NOTE: Female candidates who are prescribed a non-study-provided ARV regimen should discuss the safety of that regimen during conception and pregnancy with the prescribing physician. Such individuals should follow medical guidance regarding any potential need for contraception while using the non-study-provided ARV regimen.

4.2 **Step 1** Exclusion Criteria **for Group 1**

4.2.1 Positive HIV-1 antibody test ≥90 days prior to study entry.

4.2.2 Active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements.

4.2.3 Any acute, chronic, or recent and clinically significant medical condition that, in the opinion of the site investigator, would interfere with adherence to study requirements or jeopardize the safety or rights of the participant.

4.2.4 Receipt of an investigational study agent within 28 days prior to enrollment.

4.2.5 Chronic or recurrent use of medications that modify **the** host immune response, e.g., oral or parenteral steroids, cancer chemotherapy.

4.2.6 AHI diagnosis within 60 days **prior to study entry** after receiving any investigational ARV or HIV-1 vaccine or immune prophylaxis for HIV-1 infection.

4.2.7 **Use** of ARVs for **any reason, including** pre- **or** post-exposure prophylaxis within 60 days prior to **study entry**.

4.3 Step 2 Inclusion Criteria

NOTE: Below inclusion criteria apply to the following groups of participants:

- **Participants transitioning directly from Step 1 to Step 2 as part of A5354 Version 2.0.**

OR

- **Participants enrolling to Step 2 after having previously completed the week 72 visit and who went off study under A5354 Version 1.0.**

4.3.1 Most recent HIV-1 RNA <50 copies/mL obtained within 90 days prior to Step 2 entry.

4.3.2 Completed 72 weeks of follow-up on Step 1.

NOTE: Participants who discontinued A5354 following a full 72 weeks of follow-up on Step 1 may enroll to Step 2 regardless of time spent off-study, given they meet other eligibility criteria.

4.3.3 Ability and willingness of candidate to provide written informed consent that includes Step 2.

4.3.4 Ability and willingness to continue ART.

NOTE: Switching to the study-provided ARV or staying on the current ARV regimen prior to Step 2 entry is permitted.

4.3.5 Ability and willingness to participate in scheduled study visits per SOE.

4.3.6 Female candidates of reproductive potential who are not pregnant at the time of Step 2 enrollment and who will receive the study-provided EVG/COBI/FTC/TAF or BIC/FTC/TAF must agree not to participate in the conception process (i.e., active attempt to become pregnant, in vitro fertilization), and if participating in sexual activity that could lead to pregnancy, the female candidate must agree to use at least one reliable form of contraceptive while receiving study-provided treatment.

Female candidates are considered to be of reproductive potential if all of the following conditions apply:

- **Candidate has experienced menarche.**
- **Candidate has not undergone bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.**
- **Candidate has not experienced menopause, defined as lack of menstruation within the preceding 12 months.**

Acceptable contraceptive methods include:

- **Condoms (male or female) with or without a spermicidal agent**
- **Diaphragm or cervical cap with spermicide**
- **Intrauterine device**
- **Hormonal contraceptive**

Female candidates who are not of reproductive potential or whose male partner(s) has documented azoospermia are not required to use contraceptives. Any statement of self-reported sterility or that of her partner must be entered in the source documents.

NOTE: Acceptable documentation of lack of reproductive potential is oral or written documentation from the individual.

4.3.7 Women who are pregnant at the time of enrollment into Step 2 and agree to use a non-study-provided ARV regimen per section 8.9, and who otherwise meet requirements are eligible.

NOTE: Female candidates who are prescribed a non-study-provided ARV regimen should discuss the safety of that regimen during conception and pregnancy with the prescribing physician. Such individuals should follow medical guidance regarding any potential need for contraception while using the non-study-provided ARV regimen.

4.4 Step 2 Exclusion Criteria

NOTE: Below exclusion criteria apply to the following groups of participants:

- **Participants transitioning directly from Step 1 to Step 2 as part of A5354 Version 2.0.**

OR

- **Participants enrolling to Step 2 after having previously completed the week 72 visit and who went off study under A5354 Version 1.0.**

4.4.1 ARV interruption for greater than or equal to 7 consecutive days at any time after ART initiation and prior to entry into Step 2, including while on Step 1 or after completion of Step 1.

4.4.2 Protocol-defined virologic failure (section 6.3.4) at any time prior to entry into Step 2, including while on Step 1 or after completion of Step 1.

4.4.3 Active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements.

4.4.4 Any acute, chronic, or recent and clinically significant medical condition that, in the opinion of the site investigator, would interfere with adherence to study requirements or jeopardize the safety or rights of the participant.

4.4.5 Receipt of any investigational study agent since entry to Step 1.

4.4.6 Chronic or recurrent use of medications that modify the host immune response (e.g., oral or parenteral steroids, cancer chemotherapy, biologics).

4.5 Study Enrollment Procedures

4.**5**.1 Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol consent form approved, as appropriate, by their local institutional review board (IRB)/Ethics Committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) WILL be reviewed and approved by the DAIDS PRO, and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approval(s) for an amendment, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICF(s) WILL NOT be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual (**https://rsc.niaid.nih.gov/clinical-research-sites/daids-protocol-registration-policy-and-procedures-manual)**.

Once a candidate for study entry has been identified, details will be carefully discussed with the participant. The participant will be asked to read and sign the approved protocol consent form.

Participants from whom a signed informed consent has been obtained may be screened and enrolled, if they otherwise qualify. An ACTG Screening Checklist must be entered through the DMC Participant Enrollment System.

4.5.2 Protocol Activation

Prior to enrollment, sites must complete the Protocol Activation Checklist found on the ACTG Member website. This checklist must be approved prior to any screening of participants for enrollment.

4.**5**.3 Participant Registration

For candidates from whom informed consent has been obtained, but who are deemed ineligible or who do not enroll into the initial protocol step, an ACTG Screening Failure Results form must be completed and keyed into the database. Participants who meet the enrollment criteria will be registered to the study according to standard ACTG DMC procedures.

4.**6** Coenrollment Guidelines

- US sites are encouraged to coenroll participants in A5128, “Plan for Obtaining Informed Consent to Use Stored Human Biological Materials (HBM) for Currently Unspecified Analyses.”
- Non-US sites are encouraged to coenroll participants in A5243, “Plan for Obtaining Human Biological Samples at Non-US Clinical Research Sites for Currently Unspecified Genetic Analyses.”
- Coenrollment in A5128 or A5243 does not require permission from the A5354 protocol chairs.
- For specific questions and approval for coenrollment in other studies, sites should contact the protocol team via e-mail as described in the Study Management section.

5.0 STUDY TREATMENT

5.1 Regimens, Administration, and Duration

Study treatment is defined as STR elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (EVG/COBI/FTC/TAF) **or bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF)**, which will be provided through the study.

NOTE: Switching of ARV regimen is allowed (i.e., from EVG/COBI/FTC/TAF to BIC/FTC/TAF, or vice versa, or to an alternate non-study-provided ART) without consulting the CMC.

5.1.1 Regimen

Participants will receive elvitegravir 150 mg/cobicistat 150 mg/emtricitabine 200mg/tenofovir alafenamide 10mg (EVG/COBI/FTC/TAF) **or bictegravir 50mg/emtricitabine 200 mg/tenofovir alafenamide 25 mg (BIC/FTC/TAF)**.

NOTE: 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.

5.1.2 Administration

EVG/COBI/FTC/TAF **(with food) or BIC/FTC/TAF** (**with or without food)** will be administered as one tablet by mouth daily**.**

5.1.3 Duration

On Step 1, participants will take study medications for up to **72** weeks.

On Step 2, participants will take study medications for up to **144** weeks.

5.2 Study Product Formulation and Preparation

EVG/COBI/FTC/TAF **and BIC/FTC/TAF** tablets. Store at 25°C (77°F); excursions permitted from 15-30°C (59°-86°F); USP Controlled Room Temperature.

5.3 Pharmacy: Product Supply, Distribution, and Accountability

5.3.1 Study Product Supply/Distribution

EVG/COBI/FTC/TAF **and BIC/FTC/TAF** will be supplied by Gilead.

EVG/COBI/FTC/TAF **and BIC/FTC/TAF** will be available through the NIAID Clinical Research Products Management Center (CRPMC). The site pharmacist

should obtain the study product(s) for this protocol by following the instructions in the manual Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

Any study product not provided by the study must comply with the NIAID (DAIDS) policy that outlines the process for authorizing the use of study products not marketed in the US in NIAID (DAIDS)-supported and/or -sponsored clinical trials. This policy is available on the NIAID (DAIDS) website at **https://www.niaid.nih.gov/sites/default/files/NonFDAapprovedProducts.pdf.**

5.3.2 Study Product Accountability

The site pharmacist is required to maintain complete records of all study products received from the NIAID CRPMC and subsequently dispensed. At US CRSs, all unused study products must be returned to the NIAID CRPMC (or as otherwise directed by the sponsor) after the study is completed or terminated. The procedures to be followed are provided in the manual *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.* At non-US CRSs, the site pharmacist must follow the instructions in the *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks* for the destruction of unused study products.

5.4 Concomitant Medications

Whenever a concomitant medication or study agent is initiated or a dose changed, investigators must review the concomitant medication's and study agent's most recent package insert, Investigator's Brochure, or updated information from DAIDS to obtain the most current information on drug interactions, contraindications, and precautions.

Additional drug information may be found on the updated ACTG Drug Interactions Database located at http://tprc.pharm.buffalo.edu/home/di_search/.

5.4.1 Required Medications

Participants must be on a combination ARV regimen. The study-provided regimen will be STR EVG/COBI/FTC/TAF **or BIC/FTC/TAF**. EVG/COBI/FTC/TAF **or BIC/FTC/TAF**, but alternative regimens are allowed based on clinical indication or participant preference (section 5.1.1). **Switching of ARV regimens is allowed (e.g., from EVG/COBI/FTC/TAF to BIC/FTC/TAF or to an alternate non-study-provided ART) without consulting the CMC.**

5.4.2 Prohibited Medications

Table 5.4.2-1: Prohibited Medications for EVG/COBI/FTC/TAF

Medication/Drug Class	Prohibited Medication
Anti-infectives	**Any anti-HIV combination drugs containing FTC or lamivudine such as** • **Atripla (efavirenz, emtricitabine, and tenofovir);** • **Combivir (lamivudine and zidovudine);** • **Complera (rilpivirine, emtricitabine, and tenofovir);** • **Epivir (lamivudine);** • **Epzicom (abacavir and lamivudine);** • **Stribild (cobicistat, elvitegravir, emtricitabine, and tenofovir);** • **Trizivir (abacavir, lamivudine, and zidovudine); and** • **Truvada (emtricitabine and tenofovir).**
Concomitant use with Tybost and atazanavir or darunavir AND any of the following drugs listed below:	
Alpha 1-Adrenoreceptor Antagonist	• **Alfuzosin**
Antiarrhythmic	• **Dronedarone**
Anti-bacterial	• **Rifamycins (rifampin, rifabutin, rifapentine)**
Anti-infectives	**Any anti-HIV drugs containing tenofovir:** • **Atripla;** • **Complera;** • **Descovy** • **Odefsey** • **Stribild; and** • **Symtuza** • **Truvada** **In combination with HEPSERA**
Antimicrobial	• **Rifampin**
Antineoplastics	• **Irinotecan**
Ergot Derivatives	• **Dihydroergotamine,** **Ergotamine,** **Methylergonovine**
GI Motility Agent	• **Cisapride**
Herbal Products	• **St. John's wort** **(*Hypericum perforatum*)**
HMG-CoA Reductase Inhibitors	• **Lovastatin,** **Simvastatin**
Nephrotoxic drugs	• **Cidofovir** • **Hepsera (adefovir)**
Non-nucleoside Reverse Transcriptase Inhibitor	• **Nevirapine**
Phosphodiesterase-5	• **Sildenafil when administered as Revatio for the treatment of**

Medication/Drug Class	Prohibited Medication
(PDE5) Inhibitor	**pulmonary arterial hypertension**
Protease Inhibitor	• **Indinavir**
Sedative/Hypnotics	• **Triazolam, chronic oral midazolam (one oral dose permitted)**
Due to the need to use Vitekta with a protease inhibitor coadministered with ritonavir, consult prescribing information of coadministered protease inhibitor and ritonavir for their contraindications.	
Anti-infectives	• **Any anti-HIV drugs containing elvitegravir:** • **Stribild**

Table 5.4.2-2: Prohibited Medications for BIC/FTC/TAF

Medication/Drug Class	Prohibited Medication
Antiarrhythmic Agent	**Dofetilide**
Anticonvulsants	• **carbamazepine** • **oxcarbazepine** • **phenobarbital** • **phenytoin**
Antimycobacterials	• **rifabutin** • **rifampin** • **rifapentine**
Antiretrovirals	• **Any antiretroviral drug that is not part of the study regimen**
Herbal/Natural Supplements	• **St. John's Wort**

5.4.3 Precautionary Medications

Package inserts or investigator brochures for ARV drugs and concomitant agents and updated information from the DAIDS should be consulted whenever a concomitant medication is initiated or a dose is changed. Additional drug information may be found in the ACTG Drug Interaction Database on the ACTG website at: https://actgnetwork.org/ACTG-Drug-Interactions-Database.

Table 5.4.3-1: Precautionary Medications for EVG/COBI/FTC/TAF

- **Take caution when used in patients with known risk factors for liver diseases and impaired renal function.**
- **Reduction of the dosage of Emtriva is recommended for patients with impaired renal function.**
- **Take caution when used in patients at risk for renal dysfunction and/or who have new onset or worsening renal impairment.**

<table>
<tr><td colspan="2">• Take caution when used in combination with a protease inhibitor and cobicistat.</td></tr>
<tr><td>Anticoagulants
Antifungals
Anti-inflammatory</td><td>Colchicine
Rivaroxaban
Voriconazole</td></tr>
<tr><td colspan="2">• Take caution when used in patients with medical conditions (e.g., new onset or worsening renal impairment) or who are receiving drugs needing monitoring with estimated creatinine clearance.
• Coadministration of Tybost and tenofovir DF is not recommended in patients who have an estimated creatinine clearance below 70 mL/min because dose adjustment of tenofovir DF is required below 50 mL/min and such dose adjustments have not been established for coadministration with Tybost.
• Coadministration of Tybost and tenofovir DF in combination with concomitant or recent use of a nephrotoxic agent is not recommended.</td></tr>
<tr><td colspan="2">The following ARVs are not recommended in combination with Tybost because dosing recommendations for such combinations have not been established, and co-administration may result in decreased plasma concentrations of the ARV agents, leading to loss of therapeutic effect and development of resistance:
• More than one ARV that requires pharmacokinetic enhancement (i.e., two protease inhibitors or a protease inhibitor in combination with elvitegravir)
• Atazanavir in combination with efavirenz in treatment-experienced patients
• Atazanavir in combination with etravirine
• Darunavir 600 mg twice daily
• Darunavir in combination with efavirenz, nevirapine, or etravirine
• Other HIV-1 protease inhibitors including fosamprenavir, saquinavir, or tipranavir
• Tybost in combination with Stribild fixed-dose combination tablets (elvitegravir, cobicistat, emtricitabine, tenofovir DF) is not recommended because cobicistat is a component of Stribild.
• Tybost in combination with lopinavir/ritonavir or regimens containing ritonavir is not recommended due to similar effects of Tybost and ritonavir on CYP3A.</td></tr>
</table>

Table 5.4.3-2: Precautionary Medications for BIC/FTC/TAF

Medication/Drug Class	Precautionary Medications
Medications or oral supplements containing polyvalent cations (e.g., Mg, Al, Ca, Fe): **Calcium or iron supplements** **Cation-containing antacids or laxatives** **Buffered medications**	• **Antacids containing Al/Mg or Calcium:** • **BIKTARVY and supplements containing calcium or iron can be taken together with food. Routine administration of BIKTARVY under fasting conditions simultaneously with, or 2 hours after, supplements containing calcium or iron is not recommended.** • **BIKTARVY can be taken under fasting conditions 2 hours before antacids containing Al/Mg or calcium.** • **Routine administration of BIKTARVY simultaneously with, or 2 hours after, antacids containing Al/Mg or calcium is not recommended.** • **Supplements containing Calcium or Iron:**
Hypoglycemic agent	• **Metformin. Refer to the prescribing information of metformin for assessing the benefit and risk of concomitant use of BIKTARVY and metformin.**

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of **Evaluations** (**SOE) for Step 1**

Table 6.1-1: Step 1 SOE

Evaluation	Screen**ing**	**Step 1** Entry	1	2 (section 6.3.3)	4	8 (section 6.3.3)	12	24 (section 6.3.3)	36	48	49 (optional, section 6.4.8)	60	72	Virologic Failure Confirmation	Premature Study/ Treatment Dis**continuation**
	-7 days	day 0	± 3 days		±7 days		±14 days			-14 days to +3 days	-3 days to +14 days	± 42 days			
Documentation of AHI	X														
Fiebig Staging		X													
Demographics		X													
Informed Consent	X														
Medical History	**X**	X													
Medication History	**X**	X													
Clinical Assessment		X	X		X		X	X	X	X		X	X		X
Complete Physical Exam		X													X
Targeted Physical Exam			X		X		X	X	X	X		X	X		
Height		X													
Weight		X	X		X		X	X	**X**	X		X	X		X
Signs, Symptoms, and Diagnoses		**X**	**X**		**X**		**X**	**X**	**X**	**X**		**X**	**X**		**X**
Concomitant Medications			**X**		**X**		**X**	**X**	**X**	**X**		**X**	**X**		**X**
ART Modifications			**X**		**X**		**X**	**X**	**X**	**X**		**X**	**X**		**X**
ART Initiation		X													
Hematology, Chemistries, Liver Function Tests		X			X		X	X		X		X	X		X
Urinalysis		X													X

Evaluation	Screen**ing**	**Step 1** Entry	1	2 (section 6.3.3)	4	8 (section 6.3.3)	12	24 (section 6.3.3)	36	48	49 (optional, section 6.4.8)	60	72	Virologic Failure Confirmation	Premature Study/ Treatment Dis**continuation**
	-7 days	day 0	± 3 days		±7 days		±14 days			-14 days to +3 days	-3 days to +14 days	± 42 days			
Pregnancy Testing (section 6.**4**.7)		X	If sus-pected		If sus-pected		Whenever pregnancy is suspected								X
CD4+/CD8+ T-cells		X					X	X		X		X	X	X	**X**
PBMC and Plasma Storage		X (section 6.4.8)			X		X (section 6.4.8)	X (section 6.4.8)		X	If needed	X (section 6.4.8)	X (section 6.4.8)		
Plasma HIV-1 RNA (section 6.4.9)		X			X		X	X	X	X		X	X	X	X
HIV-1 Genotype (section 6.4.9)		X												X	
Total HIV-1 DNA by qPCR (section 6.4.9)		X								X	If needed				
Telephone Evaluations (section 6.3.3)				X		X						X			
Adherence Assessment and Counseling			X		X		X	X	X	X		X	X	X	
Consent for Optional Procedures and Large Volume Blood Collection (after week 48; section 6.4.11)		X										X			
Leukapheresis (optional as an alternative to large volume blood draw; (section 6.4.11)		X													
Gut Biopsy (optional; section 6.4.11)												X			
Lumbar Puncture (optional; section 6.4.11)												X			

6.2 SOE for Step 2

Table 6.2-1: Step 2 SOE

Evaluation	Step 2 Registration	Post-Step 2 Registration Evaluations (Weeks)	Virologic Failure Confirmation	Premature Study/Treatment Discontinuation
	Day 0	Q24 (24, 48, 72, 96, 120, and 144) ± 60 days		
Clinical Assessment	X	Q24		X
Complete Physical Exam	X			X
Targeted Physical Exam		Q24		
Weight	X	Q24		X
Confirmation of ART Continuation	X	Q24		
ART Modifications	X	Q24	X	X
Hematology, Chemistries, Liver Function Tests	X	Q24 (perform if needed, section 6.4.7)		X
Pregnancy Testing (section 6.4.7)	X	Whenever pregnancy is suspected		X
CD4+/CD8+ T-cells	X	Q48 (record results from routine care, section 6.4.8)		
Plasma HIV-1 RNA (section 6.4.9)	X	Q24	X	X
Adherence Assessment	X	Q24	X	

6.**3** Timing of Evaluations

6.**3**.1 Screening Evaluations

Screening evaluations must occur prior to the participant starting any study medications.

Screening evaluations to determine eligibility must be completed within 7 days prior to study entry unless otherwise specified.

NOTE: The screening evaluations can be combined with entry evaluations and can occur on the same day.

In addition to data being collected on participants who enroll into the study, demographic, clinical, and laboratory data on screening failures will be captured in a Screening Failure Results form and entered into the ACTG database.

6.**3**.2 Entry Evaluations

Entry evaluations must occur after the screening evaluations, unless otherwise specified, and can occur on the same day.

NOTE: **All Step 1 post-entry evaluations occur in reference to the date on which the participant initiates ART treatment. All Step 2 post-entry evaluations occur in reference to the date of enrollment into Step 2.**

Participants must immediately initiate ART at the time of **Step 1** study entry, ideally, within 24 hours but no later than 48 hours after registration/enrollment. If ART is not started within 48 hours after enrollment, the site staff should contact the protocol team immediately for approval to redraw blood samples for HIV-1 RNA and Fiebig staging and to initiate ART on the same day as redraw.

For participants who initiated ART more than 48 hours after enrollment and who did not have blood samples redrawn for HIV-1 RNA and Fiebig staging on the day of ART initiation, the site staff should contact the protocol team immediately for approval to redraw blood samples for HIV-1 RNA and Fiebig staging and to have the participants continue on ART. If allowed to remain on the study, their participation may end at **the Step 1** week 24 **visit** if deemed by the study team that they are no longer eligible for the study **based on the centralized testing results for Fiebig stage**.

Pregnancy testing should be done prior to ART initiation to inform selection of ART, but pregnancy is not an exclusion criterion for enrollment.

HIV-1 RNA testing will also be performed at the time of study entry. Results of the HIV-1 RNA test will generally be made available within 72 hours. ART

initiation will not be delayed while awaiting confirmation of the entry HIV-1 RNA results.

Results of baseline hematology, chemistry, and liver function testing will also generally be made available within 72 hours of study entry. The results of baseline laboratory evaluations and urinalysis will be reviewed with each study participant as part of the clinical assessment at week 1. ART may be dose-adjusted, otherwise altered, or discontinued if the estimated glomerular filtration rate is reduced or other medical indications for therapeutic changes are detected.

NOTE: ART must be started immediately even if the baseline safety laboratory results are not available within the 48-hour window.

If an HIV-1 resistance genotype is performed as part of clinical care during screening or any time on the study, the results of that genotyping report will be collected. The site investigator may use this information to guide a change in ART, if clinically indicated, potentially including a change from the study-provided ARV regimen to a non-study-provided ARV regimen or a change from a non-study-provided ARV regimen to the study-provided ARV regimen **or a change between the two study-provided ARV regimens**. Stored blood may be used for additional genotype testing for research purposes. This testing will not be done in real-time.

6.**3.**3 Post-Entry Evaluations

All post-entry evaluations occur in reference to the date of ART initiation.

On-ART **Step 1** Evaluations
The on-treatment visits have the following window:

- Weeks 1 and 2 (±3 days) **for all participants**
- Weeks 4 and 8 (±7 days) **for all participants**
- Weeks 12 **and** 24 (±14 days) **for all participants**
- **Week 36 (±14 days)**
- Week 48 (-14 days through +3 days)
- Week 49 (**optional, if needed to achieve blood volume collection goal at week 48;** -3 days through +14 days)
- week 60 **and** 72 (±42 days)

Step 1 Telephone Evaluations
Evaluations that are scheduled for weeks 2 and 8 **on Step 1** will be completed by the site staff via telephone (in lieu of an in-person visit by the participant) and documented on the electronic case report form (eCRF). Site staff will call the participant to inquire about recent Grade ≥2 AEs and provide adherence counseling. If any Grade ≥2 AEs that require further investigation are reported, the participant may be asked to present for in-person evaluation. Follow-up phone calls may be indicated. Site staff may use the telephone script provided

in the A5354 MOPS as guidance and for documentation of the telephone encounter(s).

NOTE: The day following the optional gut biopsy via flexible sigmoidoscopy and/or lumbar puncture procedures to be performed between weeks 60 and 72, site staff will contact the participants via telephone to inquire how they are feeling, which will be documented on the eCRF (**refer to** section 6.**4.**11 for additional information). If any symptoms are present on the day of the initial telephone call, follow-up phone calls to the participants are recommended. Site staff must ensure that the follow-up phone calls are completed as part of the **follow-up of optional procedure** evaluations.

Step 2 Evaluations

The Step 2 Registration evaluation may be combined with the Step 1 Week 72 visit. If the Step 2 Registration visit is not combined, then all Step 2 evaluations must be performed within 2 weeks after the final Step 1 visit. Those participants re-enrolling into the study after going off study at week 72 under protocol Version 1.0 will complete all evaluations listed in the Step 2 Registration column.

All visits following Step 2 registration will have a ± 60-day visit window.

Study Completion Evaluations

The **week 24 visit on Step 1** will be the final study visit for participants who initiated ART during Fiebig VI **(based on confirmed Fiebig stage results), and these participants will be replaced. For Step 2, the final visit will occur at week 144 for all the study groups, except in cases of premature study discontinuation as described in section 6.3.5.** Upon study completion, ART will no longer be provided through the study (i.e., post-trial access to ART is not within the scope of A5354). The study team will ensure that participants are referred to appropriate health facilities to continue their ART. After the study ends, ART regimens may be modified according to local practices and decision by the participants and their physicians.

6.**3**.4 Confirmation of Virologic Failure (VF) Evaluation **on Step 1 and Step 2**

VF is defined as having two consecutive HIV-1 RNA >200 copies/mL 24 **weeks or more after study entry,** or at any time after achieving HIV-1 RNA <50 copies/mL. A confirmatory plasma HIV-1 RNA measurement **and other evaluations per sections 6.1 and 6.2** should be done as soon as possible, ideally within 7 to 14 days of the initial sample with a HIV-1 RNA >200 copies/mL. If the visit for HIV-1 RNA confirmation coincides with a regularly scheduled visit, the evaluations should be combined. **Refer to** section 6.3.5 for instructions on the discontinuation evaluations for participants who have confirmed VF. **Participants who interrupt ART for ≥7 consecutive days prior to meeting criteria for VF should be managed per section 6.3.6.**

NOTE: If genotyping is performed as part of routine care, then the results will be documented in the eCRF. Stored blood may be used for additional genotype testing for research purposes.

6.**3**.5 Discontinuation Evaluations

Evaluations for Registered Participants Who Do Not Start ART
For participants who do not initiate ART per section 6.3.2, all eCRFs must be keyed for the period up to and including the entry visit. **These participants are not required to complete any study discontinuation visit. These participants will be replaced.**

Evaluations for Registered Participants Found NOT to Have HIV-1 Infection
Participants who initiated ART and are subsequently determined not to have HIV-1 infection will stop all ART, undergo a study discontinuation visit **(refer to sections 6.1 and 6.2 for discontinuation evaluations)**, and discontinue study follow-up (**refer to** section 6.4.9 for the HIV-1 RNA detectable threshold requirement). These participants will be replaced.

Premature Study Discontinuation Evaluations
Participants who are confirmed to have VF or prematurely discontinue from the study **for other reasons** will have the study discontinuation evaluations performed **(per sections 6.1 and 6.2) within 12 weeks of being notified of the need for study discontinuation**. **This may include recruitment for participation in future studies that enroll participants who initiated ART during AHI. In such cases, A5354 study discontinuation evaluations should be performed at or before enrollment into the new study.**

6.3.6 ART Interruption

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 interruption lasting ≥7 consecutive days), then the participant will be withdrawn from the study and undergo the premature study discontinuation evaluation (section 6.3.5), after which study drug will no longer be provided. This visit should occur by week 24 on Step 1, or within 12 weeks of being notified of the need for study discontinuation, whichever is later. This timeline should allow the study staff and participant to identify an alternative source of medication.**

If the participant has previously been documented to have HIV-1 RNA <50 copies/mL or the ART interruption occurs at study week 24 or later in Step 1 or Step 2, then the participant will be evaluated for VF (section 6.3.4). If the confirmatory HIV-1 RNA results indicate VF, then the participant will be withdrawn from the study and undergo premature study discontinuation

evaluation. If the confirmatory HIV-1 RNA results do not indicate VF, then **the participant** will remain in the study for continued follow-up and will complete all scheduled study evaluations per **sections 6.1 and 6.2**, except **no** PBMC and plasma collection will be performed **after the collection at week 48 on Step 1 and no optional procedures at week 60 or 72 on Step 1**.

6.**4** Instructions for Evaluations

Each study site and laboratory involved in this study will comply with the DAIDS policy on Requirements for Laboratories Performing Testing for DAIDS-Supported and/or Sponsored Clinical Trials, which is available at: https://www.niaid.nih.gov/sites/default/files/laboratorypolicy1.pdf.

All clinical and laboratory information required by this protocol is to be present in the source documents. Sites must refer to the Source Document Guidelines on the DAIDS website for information about what must be included in the source document: **https://www.niaid.nih.gov/sites/default/files/daids-sourcedocpolicy.pdf**.

All stated evaluations are to be recorded on the eCRF unless otherwise specified. Refer to section 7.0 for information on the **Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table),** and AE reporting **of adverse events requirements**.

6.**4**.1 Documentation of AHI

Section 4.1.1 specifies assay requirements for documentation of the diagnosis of AHI by local laboratories per standard of care. HIV-1 documentation will be recorded on the eCRF.

6.**4**.2 Fiebig Staging

Characterization of Fiebig stage using samples collected at the time of study entry will be performed by a designated central laboratory, and results will be available within 12 weeks after enrollment, which will help determine total follow-up period in the study (section 6.**3**.2 for details). Fiebig staging will be assigned according to the following table:

Table **6.4.**2-**1.** Fiebig Staging Assignment by Study Group

Study Group*	Fiebig Stage	Markers				
		HIV-1 RNA	and/or	IgM	and/or	WB or Geenius HIV-1/HIV-2
1	I/II	+	And	-		-
2	III/IV	+	And	+	and	-/indeterminate
3	V	+	And	+/-	and	+ without p31 band
	VI	+	And	+/-	and	+ with p31 band

Adapted from Fiebig AIDS 2003

Refer to section 6.4.9 for additional information and the A5354 Laboratory Processing Chart (LPC) for instructions related to the collection, processing, and shipping of these samples.

6.**4**.3 Demographics

Some socio-demographic information (e.g., substance use, education) will be documented on the eCRF.

6.**4**.4 Medical History

The medical history must include all diagnoses identified by the ACTG criteria for clinical events and other diagnoses. In addition to reporting all diagnoses within the past 30 days, the following diagnoses should be reported regardless of when the diagnosis was made:

- **Acute HIV-1**
- Bone fractures (verbal history accepted)
- Coronary heart disease
- Cancer (exclusive of basal/squamous cell skin cancer)
- Diabetes
- Tuberculosis
- Chronic hepatitis C
- Chronic hepatitis B
- Renal disease

Any allergies to any medications and their formulations must also be documented.

6.**4**.5 Medication History

A medication history must be present, including start and stop dates. The table below lists the medications that must be included in the history.

Table **6.4.5-1:** Medication History

Medication Category	Complete History or Timeframe
ART	Complete history
Immune-based therapy	Within 90 days prior to study entry
HIV-1-related vaccines	Within 2 years prior to study entry
Drugs for treatment of opportunistic infections	Within 90 days prior to study entry
Drugs for treatment of medical conditions Grade ≥2 as reported in the medical history	Within 90 days prior to study entry
Sex-hormone medications or sex-hormone analogues or antagonists*	**Last 12 months except as noted below**

***Includes: Hormone-releasing IUDs (e.g., Mirena inserted in the last 5 years); oral, injectable, implanted, or patch contraceptives; vaginal ring, creams, or inserts; estrogen,**

progesterone, or testosterone therapy; leuprolide or other synthetic gonadotropin-releasing hormone; tamoxifen, raloxifene, aromatase inhibitors, or any other androgen, estrogen, or progesterone analogue or antagonist therapy.

6.**4**.6 Clinical Assessments

The results of baseline laboratory evaluations and urinalysis will be reviewed with each study participant as part of the clinical assessment at week 1.

Complete Physical Exam
At entry and the premature study/treatment discontinuation visits, a complete physical examination is to include at a minimum an examination of the skin, head, mouth, and neck; auscultation of the chest; cardiac exam; abdominal exam; and examination of the lower extremities for edema. The complete physical exam will also include signs and symptoms, diagnoses, and vital signs (temperature, pulse, and blood pressure per local standard of care).

Targeted Physical Exam
At the post-entry visits, a targeted physical examination is to include vital signs (temperature, pulse, and blood pressure) and is to be driven by any previously identified or new signs or symptoms, including diagnoses that the participant has experienced since the last visit.

Post entry, refer to section 8.9 for collection requirements for pregnancy.

Height
Height will be recorded **per the SOE**.

Weight
Weight will be recorded **per the SOE.**

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. Please refer to section 7.2 for AE reporting requirements for post-entry signs and symptoms.

At Step 2 entry, record SAEs and chronic medical conditions per section 6.4.4 for re-enrolled participants who completed the study under protocol Version 1.0.

Diagnoses
At **Step 1** entry, all diagnoses that are described in section 6.4.4 must be recorded as medical history. Please refer to section 7.2 for AE reporting requirements for post-entry diagnoses.

At Step 2 entry, record SAEs and chronic medical conditions per section 6.4.4 for re-enrolled participants who completed the study under protocol **Version 1.0.**

Concomitant Medications
For post-entry, record new or discontinued concomitant medications for prophylaxis and treatment of opportunistic infections and other medical conditions since the last study visit, including actual or estimated start dates and stop dates.

ART Modifications
All modifications to ARV medications including initial doses, participant-initiated and/or protocol-mandated interruptions **(that are ≥7 days)**, modifications, permanent discontinuations, and last doses on study will be recorded.

In Step 2, all modifications to ARV medications will be recorded.

ART Initiation
ART must be initiated at the time of study entry, ideally, within 24 hours but no later than 48 hours after registration/study entry. If ART is not initiated within 48 hours after enrollment, and the participant remains eligible based on at least one inclusion criteria in section 4.1.1, the site staff should contact the protocol team immediately for approval to redraw blood samples for HIV-1 RNA and Fiebig staging and to initiate ART on the same day as redraw.

For participants who initiated ART more than 48 hours after enrollment and who did not have blood samples redrawn for HIV-1 RNA and Fiebig staging on the day of ART initiation, the site staff should contact the protocol team immediately for approval to redraw blood samples for HIV-1 RNA and Fiebig staging and to have the participants continue on ART. If allowed to remain on the study, their participation may end at week 24 if deemed by the study team that they are no longer eligible for the study.

6.**4**.7 Laboratory Evaluations

At **Step 1 entry and Step 2 registration**, 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.

Please refer to section 7.2 for AE reporting requirements for abnormal laboratory findings.

NOTE: Creatinine clearance should be graded using the categorical mL/min values from the DAIDS AE Grading Table, 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.

Hematology, Chemistries, and Liver Function Tests

- Hemoglobin or hematocrit, white blood cells, and platelets.
- Serum creatinine will be tested and used to calculate CrCl using the Cockroft-Gault equation. Refer to the calculator located on the FSTRF website.

AST and ALT
Other laboratory tests for the optional procedures between weeks 60 and 72:

- Prothrombin time/partial prothrombin time for the optional procedures
- Complete blood count for the optional gut biopsy

NOTE: Refer to the A5354 MOPS for additional information on these laboratory tests for the optional procedures.

At **Step 1** entry, the safety laboratory results for CrCl, AST, and ALT will generally be available to the site within 72 hours after study entry and ART initiation. If necessary, the ARV regimen will be immediately adjusted upon recognition of relevant laboratory abnormalities, and site staff must notify the CMC of the adjustment in ART. The results of baseline laboratory evaluations will be reviewed with each study participant as part of the clinical assessment at week 1.

In Step 2, record the results of the safety laboratory tests, if available as part of routine care. Perform only if not done and available as part of routine care.

Urinalysis
Blood, glucose, leukocyte esterase, pH, and protein. Reflex to microscopic urinalysis if dipstick result is abnormal. **Urinalysis will be performed per section 6.1.** At **Step 1** entry, the safety urinalysis result will generally be available to the site within 72 hours after study entry and ART initiation. The results of baseline urinalysis will be reviewed with each study participant as part of the clinical assessment at week 1.

Pregnancy Test
For women of reproductive potential: urine β-HCG (urine test must have a sensitivity of 15-25 mIU/mL) or serum β-HCG test may be performed. Pregnant women who meet the eligibility criteria are allowed to enroll in the study. **Record pregnancy and pregnancy outcome per section 8.0.**

At **Steps 1 and 2** entry, the pregnancy test is to be performed, and the results must be available at the site prior to the first dose of ART in order to inform the appropriate selection of ART.

Pregnancy testing post-entry will be performed if pregnancy is suspected. Participants who have positive pregnancy test results post-entry will remain on

study for continued follow-up. **Pregnant participants will be switched from the study-provided ARV regimen to an alternative ART. Refer to** section 8.9 for information on the management of participants who are pregnant at **study** entry or become pregnant while on study.

6.**4.**8 Immunologic Studies

CD4+/CD8+ T-cells
Obtain absolute CD4+/CD8+ T-cell count and percentages during the study from a laboratory that possesses a **CLIA certification or equivalent (US sites) or IQA certification (non-US sites)**.

In Step 2, record the results from the laboratory tests (if available) that are performed as part of routine care. Do not perform if results are not available from routine care.

HIV-1-Specific T-Cell Responses
PBMC and plasma will be collected, frozen, and stored for studies of HIV-1-specific T-cell responses. In addition, the PBMC samples will be shipped in batch for HLA typing and CCR5 delta-32 genotyping at the end of the study. Please refer to the A5354 LPC for instructions related to the collection, processing, and shipping of these samples.

Participants will have **blood collected for PBMC and plasma storage at the visits indicated in the SOE. At the Step 1 entry visit, PBMC and plasma will be obtained from large volume blood collection (or the optional leukapheresis, as an alternative to the large volume blood draw).**

At week 49 (if needed), blood collected may be pooled with, or tested in lieu of, blood collected at Step 1 week 48 to obtain a sufficient quantity for any assays.

At weeks 60 and 72 **on Step 1**, **PBMC and plasma storage will be obtained**.

For participants who started ART during Fiebig VI, PBMC and plasma storage will not be performed at weeks 12 and 24 **after Step 1 entry**.

For participants who interrupt ART for ≥7 consecutive days before **Step 1** week 48 of the study, their last PBMC and plasma storage will be at week 48 **on Step 1**. If such interruption occurs after week 48, no PBMC and plasma storage will be performed at future visits.

6.**4.**9 Virologic Studies

Plasma HIV-1 RNA
At **Steps 1 and 2** entry, a real-time HIV-1 RNA test will be performed. For HIV-1 antibody negative participants who do not have documented HIV-1 RNA results

at screening but meet the AHI inclusion criteria (section 4.1.1, part **b or c),** the plasma HIV-1 RNA result at **Step 1** entry must be **≥**500 copies/mL to be considered truly positive for HIV-1 infection. If the entry HIV-1 RNA result does not meet this detectable threshold value, the participant will be discontinued from the study.

For post-entry HIV-1 RNA (real-time) evaluations, the laboratory must be certified by the DAIDS Virology Quality Assurance (VQA) Program.

Fiebig Stage
At entry, additional plasma samples will be collected and shipped to the designated central laboratory for testing to determine the Fiebig stage of participants. Please refer to the A5354 LPC for instructions related to the collection, processing, and shipping of these samples.

HIV-1 Genotype
Samples will be collected at the time points indicated in the SOE and shipped to the designated central laboratory. Sites should refer to the A5354 LPC for instructions related to the collection, processing, and shipping of these samples.

Genotyping at entry and at the time of VF may be performed on stored samples for research purposes; therefore, results will not be available in a timely manner for clinical management. Entry plasma samples will be tested for reverse transcriptase (RT) and protease (PR) resistance in those participants who did not have baseline genotype performed as part of standard of care. If genotyping is performed per standard of care, sites should document the results in the eCRF.

For participants who experienced VF on study, a plasma sample will be collected for later genotyping. Samples for genotyping can either be from the initial visit with the HIV-1 RNA rise or at the time of confirmation of VF. Samples with HIV-1 RNA above 1000 copies/mL are preferred for genotyping. Resistance testing will include RT, PR, and integrase resistance testing. A plasma sample collected at entry from these same participants will be forwarded for integrase resistance testing. These genotypes are being performed for research purposes, and results will not be available in a timely manner for clinical management. Therefore, sites should follow local standard practice regarding real-time genotyping at AHI diagnosis and at the time of VF.

NOTE: If an HIV-1 resistance genotype is performed as part of clinical care during screening or any time on the study (section 6.**3**.2), the site investigator may use this information to guide a change in ART, if clinically indicated, including a change from the study-provided ARV regimen to a non-study-provided ARV regimen or a change from a non-study-provided ARV regimen to the study-provided ARV regimen.

Total HIV-1 DNA
CAHD will be assayed by qPCR in 2 million CD4+T-cells from the **Step 1** entry time point **in participants who have undetectable CAHD at week 48** to ensure that each participant's viral DNA can be amplified by the qPCR assay. This will exclude false negative results for HIV-1 DNA detection at the primary endpoint (week 48) resulting from PCR primer mismatch or other inhibitors of PCR in the participant's sample.

Total HIV-1 DNA will be obtained either from the stored PBMC and plasma or large volume blood collection (or optional leukapheresis).

Primary Endpoint Determination
Please refer to the A5354 LPC for instructions related to the collection, processing, and shipping of these samples.

6.**4**.10 Adherence Assessment **and Counseling**

Adherence to ART will be evaluated by self-report at all visits **after study entry.** Counseling to promote adherence to ART will be provided by site staff at all visits **after study entry**.

The adherence eCRF is posted on the DMC Portal in the Forms Management Utility.

6.**4**.11 Optional Procedures and **Large Volume Blood** Collection

Based on the primary endpoint determination results (after week 48), you may be asked to provide consent for the optional procedures to be performed between weeks 60 and 72.

Participants will be encouraged to undergo at least one or both of the following procedures on Step 1:
- **Gut Biopsy**
- **Lumbar Puncture**

The following conditions will render a participant ineligible from participating in the optional procedures:
- **Pregnancy**
- **ART interruption ≥7 consecutive days at any point on Step 1**
- **Virologic failure**

Telephone follow-up will be performed by site staff the day following the gut biopsy and/or lumbar puncture to inquire about any AEs from the procedure. If any symptoms potentially related to the recent research procedure are present on the day of the initial telephone call, follow-up phone calls or in-person visits (depending upon the type of symptoms or signs) are recommended. If any AEs

that require further investigation are reported, the participant should be asked to present for in-person evaluation by a site clinician. Events meeting the reporting requirement in section 7.1 must be recorded in the eCRF.

Large Volume Blood Collection OR **Optional** Leukapheresis
A large volume blood collection for plasma and PBMC storage will take place at the Step 1 entry visit. Sites may choose to offer leukapheresis as an alternative to the large volume blood draw at Step 1 entry.

NOTE: Sites will not be reimbursed for the leukapheresis **performed at Step 1 entry**. Only the routine PBMC and plasma collection or large volume blood draw will be covered at this visit.

Refer to the A5354 LPC for instructions related to the processing, shipping, and storage of these samples.

Gut Biopsy
Gut biopsy specimens via flexible sigmoidoscopy will be collected at any time **on Step 1** from week 60 to week 72 in participants who **consent** to this procedure. **Please refer to the A5354 LPC for instructions related to the processing, shipping, and storage of these samples.**

NOTE: Participants must abstain from receptive anal intercourse for 3 days before and 7 days after the biopsy.

Lumbar Puncture
CSF will be collected via lumbar puncture **and** will be performed at any time **on Step 1** from week 60 to week 72 in participants who **consent** to this procedure. **Please refer to the A5354 LPC for instructions related to the processing, shipping, and storage of these samples.**

7.0 ADVERSE EVENTS AND STUDY MONITORING

7.1 **Definition of** Adverse Events

An adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or diagnosis that occurs in a study participant during the conduct of the study REGARDLESS of the attribution (i.e., relationship of event to protocol imposed intervention). This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition.

All AEs must be reported in the clinical database if the reporting criteria have been met for any diagnoses, signs and symptoms, and abnormal laboratory findings as described below. AEs that meet the definition of a serious adverse event (SAE) or expedited adverse event (EAE), as defined in section 7.3, must also be recorded.

7.2 Adverse Event **Collection** Requirements

Post-entry, all AEs must be reported on the eCRFs if the reporting criteria have been met.

- **All Grade ≥3 signs and symptoms**
- **All Grade ≥2 laboratory findings**
- **All targeted diagnoses (refer to the list on the A5354 PSWP)**
- **All AEs that led to a change in study treatment/intervention regardless of grade**
- **All AEs meeting SAE definition or EAE reporting requirement**

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

NOTE: SAEs **or events meeting EAE reporting requirements** should **also** be entered into the DAIDS Adverse Experience Reporting System (DAERS)**, an Internet-based reporting system**.

All AEs that are reported must have their severity graded. To grade AEs, sites must refer to the 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.

Serious Adverse Events **(SAEs)**
An SAE is defined as any untoward medical occurrence that:

- **Results in** death
- **Is** life-threatening
- **Requires** inpatient hospitalization or prolongation of existing hospitalization
- **Results in** persistent or significant disability/incapacity
- **Is a** congenital anomaly/birth defect
- **Is an** important medical event **that** may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the events listed above.

7.3 Expedited Adverse Event **(EAE)** Reporting to DAIDS

7.3.1 **Expedited** Reporting **of** Adverse Event**s** to DAIDS

Requirements, definitions and methods for expedited reporting of AEs are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the RSC website at **https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids.**

The DAIDS Adverse Experience Reporting System (DAERS), an Internet-based reporting system, must be used for EAE reporting to DAIDS. In the event of system outages or technical difficulties, **EAEs** may be submitted **using**

the DAIDS EAE Form. For questions about DAERS, please contact **NIAID CRMS Support at** CRMSsupport@niaid.nih.gov. **Please note that** site queries may also be sent from within the DAERS application itself.

Sites where DAERS has not been implemented will submit expedited AEs by documenting the information on the current DAIDS EAE Form. This form is available on the RSC website: **https://rsc.niaid.nih.gov/clinical-research-sites/paper-eae-reporting**. For questions about EAE reporting, please contact the RSC (DAIDSRSCSafetyOffice@tech-res.com).

For questions about **expedited** reporting, please contact the **DAIDS** RSC **Safety Office at** (DAIDSRSCSafetyOffice@tech-res.com).

7.3.2 Reporting Requirements for this Study

- The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study.

- The study agent**s** for which expedited reporting is required **are** EVG/COBI/FTC/TAF **and BIC/FTC/TAF**.

7.3.3 Grading Severity of Events

The DAIDS AE Grading Table, **corrected** Version 2.**1**, **July 2017**, must be used and is available on the DAIDS RSC website at **https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables**.

7.3.4 Expedited AE Reporting Period

- The expedited AE reporting period for this study is as per the Version 2.0 of the EAE Manual.
- After the protocol-defined **EAE** reporting period, unless otherwise noted, only suspected, unexpected serious adverse reactions (SUSARs), as defined in Version 2.0 of the **DAIDS** EAE Manual, will be reported to DAIDS if the site staff become aware of the events on a passive basis (from publicly available information).

7.4 **Study** Monitoring

Refer to section 10.5.

8.0 CLINICAL MANAGEMENT ISSUES

Criteria for participant management, dose interruptions, modifications, and discontinuation or changes will be mandated for toxicities attributable to the study-

provided drug, EVG/COBI/FTC/TAF **or BIC/FTC/TAF**. Participants who are receiving any non-study-provided ARV regimen should have toxicities managed for that drug according to the standard of care. NOTE: Site investigators are encouraged to consult the A5354 CMC for management of ART toxicities regardless of the ARV regimens being used.

Every effort should be made to retain participants on study as long as participant safety is maintained. If treatment-limiting drug toxicity occurs, site investigators working closely with primary care providers are encouraged to choose an alternative site-provided ART regimen based on their clinical assessment, standard of care at their site, and participant preference. Such regimen changes do not necessitate discontinuation of study participation. The A5354 CMC must be notified by e-mail regarding study drug toxicities that result in a change in regimen or discontinuation (actg.cmcA5354@fstrf.org).

If there is a treatment-limiting toxicity resulting from STR EVG/COBI/FTC/TAF **or BIC/FTC/TAF**, then the individual formulations of any component drug may be given along with a drug substitution, if necessary. However, the individual formulations will not be provided through the study.

The DAIDS AE Grading Table, **corrected** Version 2.**1**, **July 2017**, will be used for the grading of drug toxicities and is available on the DAIDS **RSC** website at **https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.**

8.1 Toxicity

The general guidelines presented in the subsequent sections below apply to toxicities that are not specifically addressed further in the DAIDS AE table. Because the goal of this study is to maximally reduce HIV-1 reservoir size with early treatment, continued viral suppression on ART is critical. **For any ongoing medical condition present prior to study entry and unrelated to ART, the study ART may be initiated provided that the site investigator deems it is in the best interest of the study participants.**

With the occurrence of AEs that are probably, possibly, or definitely related to the participant's ART, every attempt should be made to switch to safe alternative regimens. However, if there are no safe alternative regimens or the treating physician deems that it is in the participant's best interest to discontinue all ART, the ART must be promptly stopped. Participants who interrupt ART for ≥7 consecutive days must have HIV-1 RNA repeated as soon as possible and evaluated for possible VF (**refer to** section 6.**3**.4).

8.1.1 Grade 1 or 2

Participants who develop a Grade 1 or 2 AE or toxicity should continue ART unless deemed unsafe, and further management will be at the discretion of the site investigator.

8.1.2 Grade 3

If the site investigator has compelling evidence that the AE has NOT been caused by the participant's ART, dosing may continue and further management will be at the discretion of the site investigator. If the toxicity is thought to be related to ART, the regimen should be held or switched to site-provided alternative ART at the discretion of the site investigator, with the goal being to maximize tolerability and minimize time off ART. Medications other than EVG/COBI/FTC/TAF **or BIC/FTC/TAF** will not be provided by the study protocol. Regardless of whether therapy is continued, stopped, or switched, the participant should be re-evaluated closely, preferably weekly, until the AE returns to Grade ≤2. Consultation with the A5354 CMC is encouraged if there are questions about appropriate management.

NOTE: The abnormal laboratory result(s) must be back to an acceptable level, as judged by the site investigator, prior to restarting therapy for any toxicity that led to a drug substitution or discontinuation.

If AE returns to Grade ≤2 after regimen discontinuation or switch and original ART is restarted with recurrence of the same Grade 3 AE within 4 weeks, the implicated drug(s) should be permanently discontinued. If the same Grade 3 AE recurs after 4 weeks but is not thought to be related to the ART, the management scheme outlined above may be repeated. Participants experiencing Grade 3 AEs requiring permanent discontinuation or modification of ART should be followed closely until resolution of the AE.
Participants with Grade 3 asymptomatic abnormalities in hemoglobin, hematocrit, white blood cells, platelets, creatinine phosphokinase, or liver enzymes may continue study drugs.

8.1.3 Grade 4

Participants who develop a Grade 4 symptomatic AE or toxicity **that is** attributed to **the study-provided** ART must have their ART discontinued or switched to an alternative regimen if felt appropriate by the site investigator. After modification of regimen, participants should be followed closely until resolution of the AE (to a Grade ≤2 or a level deemed acceptable by the site investigator), after which if treatment was discontinued it may be restarted with substitution of another drug. Those who require ART interruption for ≥7 consecutive days must have HIV-1 RNA repeated as soon as possible and evaluated for possible VF. **Details on ART interruption are described in section 6.3.6.**

Participants with Grade 4 asymptomatic laboratory abnormalities not specifically addressed below may continue ART if the site investigator has compelling evidence that the toxicity is NOT related to the regimen.

Participants with Grade 4 asymptomatic abnormalities in hemoglobin, hematocrit, white blood cells, platelets, or creatinine phosphokinase may continue study-provided drug.

8.2 Rash

NOTE: In the event that Grade 2, 3, or 4 rash fails to resolve, increases in severity, is associated with systemic (**e.g.,** fever) or allergic (e.g., urticaria) symptoms or Grade 3 or 4 LFT elevations, or is associated with exfoliative dermatitis or mucous membrane involvement or erythema multiforme, or suspected Stevens-Johnson syndrome or necrosis requiring surgery, all ART and other potentially implicated medications must be discontinued until symptom resolution. The A5354 CMC must be consulted.

8.2.1 Grade 1 or 2

If the rash is considered to be likely due to concomitant illness or drug, standard management, including discontinuation of the likely causative agent, should be undertaken. If no other causative factor is found after clinical evaluation, the participant should be treated symptomatically until the rash resolves. Antihistamines, topical corticosteroids or antipruritic agents may be prescribed. The participant should be advised to contact the site staff immediately if there is any worsening of the rash. It is extremely important to closely monitor the participant to ensure that symptoms do not worsen. **A**dditional guidance to avoid drug interactions **is described in section 5.4**.

8.2.2 Grade 3

If the rash is thought to be related to an ART agent, participants should discontinue their drug. An alternative ART regimen may be substituted with the goal of maximizing tolerability and minimizing time off ART. The A5354 CMC must be consulted regarding management.

8.2.3 Grade 4

Participants should discontinue all ART and other potentially implicated medications. The A5354 CMC must be consulted regarding management.

8.3 Diarrhea

Symptomatic treatment of diarrhea, regardless of grade, with oral antidiarrheal drugs, or modification of ART with site-provided medications is permitted at the discretion of the site investigator. If Grade ≥3 diarrhea thought to be related to current ART persists despite symptomatic management, then the regimen should be held or modified with site-provided medications at the discretion of the site investigator until it resolves to Grade ≤2. The site staff is encouraged to consult with the A5354 CMC.

8.4 Nausea/Vomiting

Nausea/vomiting, regardless of grade, may be managed with symptomatic agents such as oral antiemetics or antiemetic suppositories, or modification of ART with site-provided medications at the discretion of the site investigator. For Grade ≥3 nausea/vomiting thought secondary to ART that fails to improve on antiemetics, the regimen may be held or modified with site-provided medications until resolution to Grade ≤2. If Grade ≥3 nausea/vomiting recurs with reintroduction of initial ART, the regimen should be permanently discontinued with the option to substitute with site-provided alternative regimens at the discretion of the site investigator. The A5354 CMC must be consulted in these situations.

If nausea/vomiting thought to be related to ART, then an alternative regimen may be substituted at any time at the provider's discretion with the goal of maximizing tolerability and minimizing time off ART. The site staff is encouraged to consult with the A5354 CMC.

8.5 Liver Enzyme Elevations

8.5.1 Grade ≤3

The participant's ART may be continued for asymptomatic, isolated Grade ≤3 AST (SGOT) or ALT (SGPT) elevations at the discretion of the site investigator. Careful assessments should be done to rule out the use of alcohol, non-study drug-related toxicity, or viral hepatitis as the cause of the liver enzyme elevation.

For symptomatic Grade 3 elevations of AST (SGOT) or ALT (SGPT), the ART should be held or changed to alternative site-provided medication until toxicity returns to Grade ≤2. **However, ART may be continued for Grade 3 elevations that are unrelated to ART at the discretion of the site investigator.** If symptomatic Grade 3 elevation recurs on rechallenge with ART, the regimen should be permanently discontinued and the A5354 CMC must be consulted.

An alternative ART regimen may also be substituted at any time at the provider's discretion with the goal of maximizing tolerability and minimizing time off ART.

8.5.2 Grade 4

The ART should be held **or modified to alternative site-provided regimen at the discretion of the site investigator** for confirmed asymptomatic AST (SGOT) or ALT (SGPT) Grade 4 elevations **that are related to ART. Laboratory follow-up should be performed** until the toxicity returns to Grade ≤2. If Grade ≥3 elevation in AST (SGOT) or ALT (SGPT) recurs on rechallenge **with previously used regimen**, **these drugs** should be permanently discontinued and the A5354 CMC must be consulted.

Any symptomatic Grade 4 AST (SGOT) or ALT (SGPT) elevation **that is believed to be related to ART** should lead to permanent discontinuation of the **regimen** with immediate consultation with the A5354 CMC.

For asymptomatic and symptomatic Grade 4 AST (SGOT) and/or ALT (SGPT) elevations that are not related to ART, the ART may be continued at the discretion of the site investigator provided that it is felt to be in the best interest of the participant.

Careful assessments should be done to rule out the use of alcohol, non-study drug-related toxicity, or viral hepatitis as the cause of the Grade 4 elevation.

8.6 Headache

Analgesics, anti-migraine drugs, muscle relaxants, and other symptomatic treatment should be used at the discretion of the site investigator. If the headache is considered to be most likely due to concomitant illness or drug, standard management, including discontinuation of the likely causative agent, should be undertaken. If the headache is thought to be related to their ART, the participant should be managed at the discretion of the site investigator. The participant should be advised to contact the site investigator immediately if there is any worsening of the headache. If the regimen is discontinued, the A5354 CMC must be consulted. An alternative ART regimen may also be substituted at any time at the site investigator's discretion with the goal of maximizing tolerability and minimizing time off ART.

8.7 Hypophosphatemia

8.7.1 Grade 3

For Grade 3 hypophosphatemia, participants should be given oral supplements or foods high in phosphates, with close follow-up at the discretion of the investigator. For persistent Grade 3 hypophosphatemia despite supplementation, consideration should be given to holding any TDF or TAF-containing regimens as outlined for Grade 4 hypophosphatemia. An alternative ART regimen may also be substituted at any time at the provider's discretion with the goal of maximizing tolerability and minimizing time off ART.

8.7.2 Grade 4

For Grade 4 hypophosphatemia, hold any TDF or TAF-containing regimens and switch to an alternative site-provided drug. Participants should be given oral phosphate supplements or foods high in phosphates. Retest phosphate levels weekly until they return to Grade ≤1.

Continuation of supplemental phosphate is at the discretion of the site investigator. If Grade ≥3 hypophosphatemia persist despite discontinuation of

TDF or TAF-containing regimen and phosphate supplementation, neither TDF nor TAF-containing regimens should be reinitiated.

8.8 Renal Insufficiency

CrCl should be calculated from the serum creatinine using the Cockroft-Gault equation. Providers should consider testing for HLA-B5701 in any participant with renal insufficiency in anticipation of a potential switch of ART to an abacavir-containing regimen, if not already taking in order to maximize participant safety and minimize time off ART.

For participants prescribed study-provided EVG/COBI/FTC/TAF, any CrCl <30 mL/min or >0.4 mg/dL increase from baseline creatinine value should be confirmed by repeat testing within 1 week. If the calculated CrCl remains <30 mL/min, the regimen must be permanently discontinued and alternative regimen initiated. Participants with confirmed serum creatinine increases >0.4 mg/dL above baseline should undergo an evaluation for potential causes of decreased renal function and should have serum creatinine monitored more frequently, at the discretion of the site investigator, until serum creatinine either stabilizes or decreases to ≤0.4 mg/dL above baseline.

Management of increasing creatinine or declining CrCl on any site-provided ART should be managed per the standard of care at the discretion of the site investigator with the goal of maximizing tolerability and minimizing time off ART.

The A5354 CMC must be consulted for any discontinuation or modification of ART due to renal insufficiency.

8.9 Pregnancy and Breastfeeding

Pregnant and breastfeeding women are allowed in the study, assuming they meet other eligibility criteria, and an appropriate alternative ARV regimen is available according to local standard practice. Participants who are pregnant or breastfeeding should not be prescribed EVG/COBI/FTC/TAF **or BIC/FTC/TAF**.

Women who are diagnosed with pregnancy following enrollment and are on study-provided EVG/COBI/FTC/TAF **or BIC/FTC/TAF** or site-provided regimen not recommended in pregnancy should be switched to a regimen that is suitable for use during pregnancy and/or breastfeeding according to the local standard practice.

Incident pregnancies that occur while on study should be reported prospectively to The Antiretroviral Pregnancy Registry. More information is available at www.apregistry.com. Phone: 800-258-4263; Fax: 800-800-1052. (For studies conducted at sites outside the US: Report to The Antiretroviral Pregnancy Registry; Fax: 44-1628-789-666 or 910-246-0637; Phone: 910-679-1598.)

Pregnant women will continue on study on an alternative non-study-provided ARV regimen and will follow the schedule of **evaluations** outlined in section 6.1, with exception of the optional procedures and large volume blood collection.

For women who deliver and elect not to breastfeed or for those who complete breastfeeding, ARV regimens can be switched to study-provided EVG/COBI/FTC/TAF **or BIC/FTC/TAF**.

If a pregnant woman has completed the study prior to delivery or if she elects to prematurely discontinue from the study before the end of the pregnancy, then site staff should request permission to contact her regarding pregnancy outcomes following delivery. This permission should be explicitly documented, and the women should be contacted within 2 weeks of her due date to obtain this information.

Any intrapartum complications**, pregnancy,** and pregnancy outcome will be recorded on the eCRFs.

8.10 Lumbar Puncture

The most common complication is positional headache (12-24 hours post-procedure), believed to be due to post-procedure leakage from the puncture site.

The primary therapy for this is to lie down. The headache subsides within minutes of lying down and if the participant stays inactive over a day or two the leakage stops. In rare cases where it is prolonged, or the participant must move about, a blood patch can be injected over the site of the puncture, effectively blocking the leakage. If a pencil point needle is used, the risk of lumbar puncture headache is small (<10%) and a blood patch is rarely needed. Participant should stay hydrated and limit strenuous activities, especially lifting of heavy weights for 24 hours after lumbar puncture or until post-procedure headache resolves. Contact with the participant on the day following the procedure is recommended. Heat and over-the-counter analgesics may be used if low back pain at the site of the puncture develops. Participant should be seen if any indication of significant increased disability is experienced.

8.11 Leukapheresis

On rare occasions, an allergic reaction to the citrate used during leukapheresis will require that the procedure be stopped early. Symptoms should be treated according to the site's standard practice.

8.12 Gut Biopsy

On extremely rare occasions, the gut biopsy via flexible sigmoidoscopy may cause pain, infection, bleeding, or perforation of the intestinal tract. If such a complication occurs, the participant will have appropriate clinical management in conjunction with the participant's health care provider.

9.0 CRITERIA FOR DISCONTINUATION

9.1 Premature Study Drug Discontinuation

- Drug-related toxicity (section 8.1)
- Requirement for prohibited concomitant medications (section 5.4)
- Pregnancy and/or breastfeeding
- Request by participant to discontinue treatment
- Clinical reasons believed life-threatening by the physician, even if not addressed in the toxicity section of the protocol

9.2 **Premature** Study Discontinuation

- Never started ART
- Started ART after 48 hours after enrollment and determined by the protocol team to be ineligible for continued participation
- Does not have HIV-1 infection
- **Participant confirmed to be Fiebig VI will discontinue after week 24 in Step 1.**
- Failure by the participant to attend three consecutive study visits
- Participant has **confirmed** VF as defined in section 6.**3**.4
- **In Step 1, interrupted ART ≥7 consecutive days and has not previously demonstrated an HIV-1 RNA <50 copies/mL (prior to the interruption lasting ≥7 consecutive days)**
- Request by the participant to withdraw**, including withdrawal to enable participation in another study.**
- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the participant.
- Participant judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.
- At the discretion of the IRB/EC, NIAID, Office for Human Research Protections (OHRP), other government agencies as part of their duties, investigator, or industry supporter.

10.0 STATISTICAL CONSIDERATIONS

10.1 General Design Issues

This is a prospective, open-label study to measure the effects of early ART on the establishment of HIV-1 reservoir and HIV-1-specific immunity. Participants with AHI will have an enrollment visit and initiate ART at the time of enrollment. Each participant will be followed **on study** for up to **216** weeks **(72 weeks on Step 1 and 144 weeks on Step 2)** unless they are Fiebig VI at ART initiation in which case their last visit will be **no later than** week 24 **on Step 1**. The primary endpoint is CAHD in 5 million blood-derived CD4+ T-cells assayed by qPCR at week 48 **on Step 1**. Only participants who have not met criteria for permanent study discontinuation, have not missed more than 6

consecutive days of ART and have HIV-1 RNA <50 copies/mL at week 48 **on Step 1** will **be included in the primary efficacy analysis**. If adequate sample and analyzable data are not available from the week 48 visit, week 49 will be used instead.

Characterization of Fiebig stage using samples at the time of ART initiation will be performed with results known within 12 weeks based on standardized, centralized testing; this will define the analysis groups. **Each study group had a target enrollment of 50 participants under protocol Version 1.0. Study Groups 2 and 3 have reached their target enrollment and therefore the original inclusion criteria from protocol Version 1.0 corresponding to these groups are no longer applicable for eligibility into the study.** Because Fiebig stage for the analysis comparison will not be known until after therapy is initiated, over-enrollment based on available data at the time of entry may be implemented**.**

10.2 Outcome Measures

10.2.1 Primary Outcome Measures

10.2.1.1 CAHD per 5 million CD4+ T-cells at week 48 **on Step 1**

10.2.2 Secondary Outcome Measures

10.2.2.1 HIV-1-specific CD4+ and CD8+ T-cell responses to nef/tat/rev/vpr/vpu, gag, pol and env by flow cytometry prior to ART initiation and while HIV-1 RNA is suppressed on ART

10.2.2.2 Cell-associated HIV-1 RNA per 5 million CD4+ T-cells prior to ART initiation and while HIV-1 RNA is suppressed on ART

10.2.2.3 Cell-associated HIV-1 RNA/DNA ratio in participants with detectable CAHD prior to ART initiation and while HIV-1 RNA is suppressed on ART

10.2.3 **Other** Outcome Measures

10.2.3.1 Frequency of non-transmitted epitope escape mutants prior to ART initiation and week 48 **on Step 1**

10.2.3.2 CAHD, HIV-1-specific immune responses and single copy HIV-1 RNA in blood; CAHD in gut; and CAHD and single copy HIV-1 RNA in CSF at any time **on Step 1** from week 60 to week 72 in participants who **consent and** undergo the optional procedures and have available data

10.2.3.3 Innate immune responses such as NK cells, ADCC, and ADCP prior to ART initiation and while HIV-1 RNA is suppressed on ART

10.2.3.4 Different HIV-1 molecular forms (unintegrated HIV-1 DNA, integrated HIV-1 DNA, total HIV-1 DNA) in participants with detectable CAHD prior to ART initiation and while HIV-1 RNA is suppressed on ART

10.2.3.5 Genetic factors such as HLA typing and CCR5 delta-32 genotyping, including relationships with virologic and immunologic outcomes prior to ART initiation and while HIV-1 RNA is suppressed on ART

10.2.3.6 Integration sites prior to ART initiation and while HIV-1 RNA is suppressed on ART

10.3 Randomization and Stratification

There is no randomization or stratification.

10.4 Sample Size and Accrual

The primary study objective is to compare the primary outcome measure at week 48 **on Step 1** after ART dichotomized as: 0 versus >0 copies of CAHD in 5 million blood-derived CD4+ T-cells. The groups are expected to differ in likelihood that a participant has no detectable total HIV-1 DNA, with percentages estimated to be:
Fiebig I/II: 75%
Fiebig III/IV: 25%
Fiebig V: <5%

Forty-two (42) virologically suppressed participants at week 48 **on Step 1** per group will provide high power to detect differences between all three groups based on this primary endpoint: >95% power for Fiebig I/II versus III/IV, >95% power Fiebig I/II versus V and 80% power for Fiebig III/IV versus V (assuming 3.5% for Fiebig V participants), based on Fisher's exact test with type I error rate of 0.05 for each pairwise comparison. Table **10.4-1** below shows power for the comparison between Fiebig I/II versus III/IV with different underlying probability of undetectable cell-associated HIV-1 DNA at week 48 with 42 participants per group. The study will have moderate power (76%) to detect a difference between the two groups even with higher estimated probability in Fiebig III/IV group (35%) and lower estimated probability in Fiebig I/II group (65%).

Table **10.4-1:** Power Comparisons

	Fiebig III/IV		
Fiebig I/II	25%	30%	35%
75%	>95%	>95%	95%
70%	>95%	95%	88%
65%	95%	88%	76%

It is further assumed that 90% of participants who initiate study treatment will remain HIV-1 RNA <50 copies at week 48 for primary endpoint evaluation. Taking into account **a**

15% lost to follow-up **rate and insufficient samples at primary outcome week**, the total sample size is **196** (**a target enrollment of 50** per group).

NOTE: Groups 2 and 3 closed to enrollment under protocol Version 1.0.

The sample size increase to 196 participants is intended to aid in meeting the accrual target for Group 1 and increase the number of early-treated participants engaged in the cohort that are potentially eligible for future studies. This is an estimation of the additional number of participants that will need to be enrolled in order to fully accrue Group 1 (since group assignment is unknown at the time of enrollment), based on the current rate of participants who were estimated to be in Group 1, and determined to be in Group 1 at study entry after centralized testing.

Based on the available Fiebig staging data as of May 15, 2019, after the completion of centralized testing, 31% of participants estimated to be in Group 1 were determined to be in Group 1, while 50% were determined to be in Group 2. Group 1 has enrolled 37 participants. Group 2 had enrolled 63 participants, and Group 3 had enrolled 54 participants. Assessing the current group counts and rates, we anticipate that, with 196 participants enrolled (an additional 36 enrollments after replacing Fiebig VI and HIV negative participants), approximately 50 participants will be determined to be in Group 1.

Secondary objectives are largely exploratory without formal sample size calculation. However, based on data from the RV254 study, HIV-1-specific CD4+ T-cell responses to gag after ART are expected to be 15% in Fiebig I/II versus 30% in Fiebig III/IV versus 80% in Fiebig V which will provide 30% power for Fiebig I/II versus III/IV, >90% power for Fiebig V versus the other two groups.

It is anticipated that the study will take **36** months to **accrue** with one third of the participants accrued during the first 6 months after at least three international sites have enrolled at least one participant.

10.5 **Data and Safety** Monitoring

Accrual, baseline characteristics (including baseline laboratory values), conduct of the study (including premature study discontinuations, data availability and sample acquisition), any ART interruptions, VFs, and all reported toxicities and AEs will be monitored by the team during the study. The team will also closely monitor the Fiebig stages of the study population as defined by the standardized testing of samples obtained at ART initiation. In addition, 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.

Furthermore, the CMC or the team may, at any time it thinks appropriate, ask for the SMC to independently review all available safety data.

Since characterization of Fiebig stage using samples at the time of ART initiation will be performed with results known within 12 weeks based on standardized, centralized testing, an estimated Fiebig group at enrollment based on inclusion criteria from section 4.1.1, as shown in the table below will provide additional real-time monitoring for accruals into each study group. Each Fiebig stage from I through IV has a median duration of approximately 5 days, the Fiebig stage at 7 days prior to enrollment may be expected to advance by one or possibly two stages by the time of enrollment. Both Fiebig stage determinations (centralized tested or estimated by inclusion criteria) will be used to guide accrual once enrollment is completed in at least one Fiebig group. For instance, Group**s 2 and** 3 **have reached their** enrollment **target under protocol Version 1.0**, **thus** enrollment **is** closed for **those two groups.**

Table **10.**5**-1**. Expected Fiebig Group Based on Inclusion Criteria (section 4.1.1)

Inclusion Criteria #	Inclusion Criteria Details	Expected Fiebig Group
a	A detectable HIV-1 RNA obtained within 28 days prior to study entry AND a non-reactive HIV-1 antibody within 7 days prior to entry	Group 1 (Fiebig I-II)
b	ARCHITECT or GSCOMBO S/CO ≥10 within 7 days prior to entry AND a non-reactive HIV-1 antibody within 7 days prior to entry	Group 1 (Fiebig I-II)
c	ARCHITECT or GSCOMBO S/CO ≥1 within 7 days prior to entry AND a non-reactive HIV-1 antibody within 7 days prior to entry AND a known prior S/CO <0.5 within 90 days prior to entry	Group 1 (Fiebig I-II)
d	ARCHITECT or GSCOMBO S/CO >0.5 but <10 within 7 days prior to entry AND a non-reactive HIV-1 antibody within 7 days prior to entry AND detectable HIV-1 RNA within 7 days prior to entry	Group 1 (Fiebig I-II)

10.6 Analyses

Analysis of the primary **efficacy** will be restricted to participants who maintain HIV-1 RNA <50 copies/mL at week 48 **on Step 1** 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 VF (section 6.**3**.4). 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). Primary outcome measures 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. Each participant's primary endpoint sample will be tested twice by gag-**

and integrase-assays. For the primary outcome measure, 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. Proportion of 0 copies of CAHD will be compared pairwise between groups using Fisher's exact test. 95% confidence interval for the proportion estimated will be calculated using the exact binomial distribution. The analysis groups will be Fiebig I/II, Fiebig III/IV, and Fiebig V, as defined by the standardized testing of samples at ART initiation. **Supplemental analyses** will use Wilcoxon rank sum test to compare the primary outcome measure on the continuous scale between analysis groups**, separately for the results from the gag- and integrase assays**. We will also examine the primary outcome measures that include participants with extended periods of ART interruption (≥7 consecutive days) while 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.

Study and study treatment discontinuations (and reasons) will be summarized. In addition, use of non-study-provided ART will be summarized including reasons (if any) for participants to change from study-provided ARV regimen to non-study-provided ARV regimen.

Analysis of secondary outcome measures will use the same approach as for 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. Rank-based Spearman correlations will describe association between outcome measures.

11.0 PHARMACOLOGY PLAN

Not applicable.

12.0 DATA COLLECTION AND MONITORING

12.1 Records to Be Kept

eCRFs will be made available to sites for data entry. Participants must not be identified by name on any data submitted to the DMC. Participants will be identified by the patient identification number (PID) and study identification number (SID) provided by the ACTG DMC upon registration.

12.2 Role of Data Management

12.2.1 Instructions concerning entering study data on eCRFs will be provided by the ACTG DMC. Each CRS is responsible for keying the data in a timely fashion.

12.2.2 It is the responsibility of the ACTG DMC to assure the quality of computerized data for each ACTG study. This role extends from protocol development to generation of the final study databases.

12.3 Clinical Site Monitoring and Record Availability

12.3.1 Site monitors under contract to the NIAID will visit participating clinical research sites to review the individual participant records, including consent forms, eCRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts), to ensure protection of study participants, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect sites' regulatory files to ensure that regulatory requirements are being followed and sites' pharmacies to review product storage and management.

12.3.2 The site investigator will make study documents (e.g., consent forms, drug distribution forms, eCRFs), and pertinent hospital or clinic records readily available for inspection by the **ACTG,** local IRB**/EC**, the site monitors, the NIAID, the OHRP, **the industry supporter or designee, other local, US, and international regulatory entities** for confirmation of the study data.

13.0 PARTICIPANTS

13.1 IRB Review and Informed Consent

This protocol and the informed consent document (Appendix I) and any subsequent modifications will be reviewed and approved by the IRB or EC responsible for oversight of the study. A signed consent form will be obtained from the participant. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant, and this fact will be documented in the participant's record.

13.2 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain participant confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the ACTG, IRB/EC, NIAID, OHRP, **other local, US, and international regulatory entities** as part of their duties, or the industry supporter or designee.

13.3 Study Discontinuation

The study may be discontinued at any time by the ACTG, IRB/EC, NIAID, OHRP, or other country-specific government agencies as part of their duties to ensure that research participants are protected, or the industry supporter or designee.

14.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by ACTG policies. Any presentation, abstract, or manuscript will be made available for review by the industry supporter prior to submission.

15.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All dangerous goods and materials, including diagnostic specimens and infectious substances, must be transported using packaging mandated by CFR 42 Part 72. Please refer to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

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APPENDIX I: SAMPLE INFORMED CONSENT

DIVISION OF AIDS
AIDS CLINICAL TRIALS GROUP (ACTG)

A5354
Effect of Antiretroviral Treatment Initiated During Acute HIV-1 Infection on Measures of HIV-1 Persistence and on HIV-1-Specific Immune Responses
FINAL Version 2.0, December 31, 2019

SHORT TITLE FOR THE STUDY: A5354/EARLIER (Early ART to Limit Infection and Establishment of Reservoir)

SUMMARY

PURPOSE: **The purpose of this study is to have persons who are newly or recently infected with HIV-1 (the virus that causes AIDS) to start taking anti-HIV medicine (antiretroviral (ARV) drugs) right away. The study will look at how starting antiretroviral treatment (ART), as soon as the HIV-1 infection is found, affects the amount of HIV-1 virus and HIV-1 infection-fighting cells (CD4+ and CD8+ T-cells) in your blood. The study will also look at the amount of HIV-1 DNA (the genetic material for HIV-1) is seen in CD4+ T-cells after you have been on ART for 48 weeks.**

The study will provide elvitegravir/cobicistat/emtricitabine/ tenofovir alafenamide (EVG/COBI/FTC/TAF) and bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF). The study doctor will have you take one of these study-provided medicine or a non-study-provided ARV medication. You must be on ART while participating in this study.

NUMBER OF PARTICIPANTS: **Up to 196 people will take part in this study.**

LENGTH OF STUDY: **The study will last about 5 years for most people. There are two parts to the study. Part 1 will take about 1 year and 6 months, and Part 2 will take about 3 years.**

You may need to stay at the clinic for almost half a day at some visits. You will have up to nine study visits in the first year of Part 1; two of these visits will be done by telephone. In the next year of Part 1 of the study, you will have up to two clinic visits. In Part 2 of the

study, you will need to come for study visits two times each year for the next three years.

REQUIRED ACTIVITIES:

Blood and urine collections
At most visits, some blood will be collected from a vein in your arm. At entry and if you leave or stop the study early, you will be asked to provide a urine sample.

Special procedure
At entry of Step 1, a large amount of blood will be collected from a vein in your arm or instead of this large volume blood draw, an optional procedure called leukapheresis may be performed. The leukapheresis procedure uses a machine that allows for collection of white blood cells (infection-fighting cells in your blood). This procedure may take 1½ to 3 hours.

RISKS:

EVG/COBI/FTC/TAF and BIC/FTC/TAF have been tested in humans and are approved by the US Food and Drug Administration (FDA). There are, however, some risks when taking these drugs. Below are some common drug reactions seen with these drugs when given to people.

- **Headache**
- **Diarrhea or loose, watery stools**
- **Nausea or vomiting**
- **Abdominal pain**
- **Tiredness or weakness**
- **Rash**

If you are taking EVG/COBI/FTC/TAF, you may also experience worsening or new damage or failure to your kidney, pancreas, or liver.

If you are living with hepatitis B virus (HBV) and taking BIC/FTC/TAF, there is a possibility of an unexpected worsening of hepatitis B if you stop taking BIC/FTC/TAF.

BENEFITS:

No direct benefits should be expected from participating in this study.

OTHER CHOICES:

Instead of being in this study, you have the option of receiving care from your primary provider or other health care providers.

INTRODUCTION

You are being asked to take part in this research study for two main reasons:
1. You are **living** with **acute** human immunodeficiency virus type 1 (HIV-1, the virus that causes AIDS). **Acute infection means that you recently got HIV**.
2. You are willing and able to start taking antiretroviral therapy (ART, anti-HIV drugs) right away.

This study is sponsored by the National Institutes of Health (NIH). The doctor in charge of this study at this site is: *(site: insert name of Principal Investigator)*. Before you decide if you want to be part of this study, we want you to know more about the study.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to take part in this study, you will be asked to sign this consent form. You will get a copy to keep.

WHY IS THIS STUDY BEING DONE?

The study is being done to:
- start ART early in those recently or acutely infected with HIV-1
- see how starting ART as soon as the infection is found affects the amount of HIV-1 in your blood and how well your body fights the HIV-1 infection
- look at the amount of HIV-1 DNA (genetic material for HIV-1) seen in CD4+ T-cells (infection-fighting cells in your blood) after 48 weeks of ART
- see how early treatment for HIV affects the numbers of HIV-1 infection fighting cells (CD4+ and CD8+ T-cells) in your blood

HOW MANY PEOPLE WILL **TAKE PART** IN THIS STUDY?

Up to 196 people (men and women age 18 years and older) who have acute HIV-1 infection will take part in this study.

HOW LONG WILL I BE IN THIS STUDY?

You will be in this study between about 1 year and 6 months and about 4 years and 6 months depending on when you join.

WHAT DO I HAVE TO DO IF I AM IN THIS STUDY?

If you decide to join this study, you will first need to be screened for the study to make sure that you qualify. You will start taking ART at study entry. At study entry, a sample of your blood will be tested to confirm your HIV-1 infection. At the time of starting ART, an additional sample of

your blood will be tested to see how far along you are with your HIV-1 infection; this is called Fiebig staging. There are three study groups determined by Fiebig staging**.** Based on your medical records of HIV testing and the results from the day that you start the HIV medications, the study team will determine which study group you will be included in.

The study will provide **two tablets – each tablet** contains **3 to** 4 different drugs. Three of these drugs treat HIV, and one is a drug that increases the levels of one of the anti-HIV drugs. The names of these drugs are elvitegravir/cobicistat/emtricitabine/ tenofovir alafenamide (EVG/COBI/FTC/TAF) and **bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF). These drugs** have been approved by the US Food and Drug Administration.

If you are not able to take the study-provided **drug**, then you will be allowed to take the best ART available to you, as prescribed by your primary care doctor. These non-study-provided ART must be obtained locally.

Both you and your doctor will know which drugs you are taking. The study staff will work with you to make sure you are taking your medication correctly.

There are two parts to the study, which are described below.

If you are eligible and enter the study, you will need to come for study visits up to nine times in the first year **of Part 1 of the study;** two of these visits will be done by telephone. If during these telephone visits, the study staff determines that you should be seen in person, you will be asked to go to the clinic. In the next year **of Part 1 of the study**, you will have up to two clinic visits that will include the study staff contacting you by telephone the day after completing the optional procedures (gut biopsy and/or lumbar puncture). You will be notified by the study staff of when to expect to receive these telephone calls before you are actually called.

If after completing the visit 72 weeks (about 1 year and 6 months) after entering the study in Part 1 (under either Version 1.0 or Version 2.0), you remained virologically suppressed (having HIV-1 viral load levels <50 copies/mL), never stopped ART for 7 days or more, and did not have detectable levels of HIV-1 viral load (higher than what the study would allow), you will enter Part 2 of the study. You will be asked to come for study visits two times each year for approximately the next 3 years. Part 2 of the study has limited evaluations and no invasive procedures.

NOTE: Groups 2 and 3 closed to enrollment since the accrual goal was met under protocol Version 1.0.

Most clinic visits will last about 1 hour. The study staff will tell you how long each visit will last.

NOTES:

- If your laboratory tests from entry show that you do not qualify for the study, the study staff will tell you to stop all ART. You will be asked to complete the discontinuation evaluations, and your follow-up will end.
- After you enter the study, if your blood test shows that you have had HIV longer than expected at the time of entry, then your follow-up and HIV study treatment will end at week

24. Your doctor will tell you the results of the tests by week 12 so you will have time to pursue HIV treatment outside of the study.
- If you stop taking your ART for 7 or more days in a row, the last peripheral blood mononuclear cells (PBMC, cells separated from the blood taken from you) and plasma (the liquid part of blood taken from you) collection will be done at week 48.

You may need to come to the clinic for extra visits if you develop side effects or if you need to switch to non-study-provided ART or if the level of HIV in your blood increases after it has been undetectable. More information about the study procedures is given at the end of this consent (Attachment A). During the study, you will receive results, when they are available, from any routine tests that are done during the study.

At most visits, we will collect blood samples for routine safety labs and study-required tests. Some of your blood will be stored (with protectors of identity) and used for immunologic, resistance, and genetic testing that is required for this study.

You will get the site's usual adherence support (procedures to help you remember to take your ART).

<u>If you do not enroll into the study</u>
After signing this consent form, if you decide not to take part in this study or if you do not meet the eligibility requirements, the study team will still use some of your information. **There is some information that we collect on everyone who is screened for an ACTG study.** As part of this screening visit, some demographic (for example, age, gender, race), clinical (for example, disease condition, diagnosis), and laboratory (for example, HIV-related blood test results) information **will be** collected from you**. We also collect information on whether you use (or have used) IV drugs.**

We will collect this information even if you do not enroll in this study. This information is collected so that ACTG researchers may determine whether there are patterns and/or common reasons why people do not join a study.

<u>Extra Samples and Blood Collections</u>
Part of the entry visit includes taking a larger amount of blood (large volume blood draw) than what is collected on a typical blood draw. At some sites, blood will be collected during a medical procedure called leukapheresis, which may be performed in place of large volume blood collection at entry. For this procedure, you will need to be in a semi-reclining or reclining position for most of this time. By collecting blood using this procedure, researchers are able to get many more white blood cells than is usually possible. For additional information, see Attachment B of Appendix I.

After week 48 **on Part 1 of the study**, you may be asked whether you agree to have additional procedures done and samples collected. You will be provided more information about these additional procedures during that visit. These additional procedures are optional and include gut biopsy by flexible sigmoidoscopy and lumbar puncture (see Appendix II for additional information). You will not receive the results of these procedures because they will be done in

the future. No matter what you decide, it will not affect your participation in the study. **About 25 people participating in this study will take part in these two optional procedures.**

At non-US sites, biological specimens may be shipped and/or stored outside the country from which they were collected (e.g., sites that do not have a central testing laboratory in-country).

NOTE: If you are pregnant or become pregnant on study, these optional procedures will not be done. **If you stop taking ART for 7 days or more in a row or if you have a viral load that is confirmed to be more than or equal to 50 copies/mL, you will not be able to participate in the gut biopsy and lumbar puncture optional procedures.**

CAN I CHOOSE THE TYPES OF RESEARCH THAT MY SAMPLES AND INFORMATION ARE USED FOR?

Some of your blood will be stored and used for study-required immunologic and virologic testing.

Identifiers will be removed from your samples and from any private information that has been collected about you. This means that no one looking at the labels or at other information will be able to know that the samples or information came from you.

The tests described above are required by this study. If you do not agree to the storage or testing that has been described above, you should not join this study.

Some blood that is collected from you during the study might be left over after all required study testing is done. This blood will be stored and, if you give your consent below, may be used for ACTG-approved research. This means that researchers who are not part of the protocol team may use your samples without asking you again for your consent. As noted above, none of your samples will have any private information about you on their labels.

You may decide whether this "extra" blood may be stored and, if so, whether additional testing may be performed on it. You may withdraw your consent for research on your extra samples at any time and the specimens will be discarded.

You will not be paid for your samples. Also, a researcher may make a new scientific discovery or product based on the use of your samples. If this happens, there is no plan to share any money with you. The researcher is not likely to ever know who you are.

At this time, we do not know whether any of the research will include testing of your genes or your DNA (your own genetic information). We do not know whether a type of testing called whole genome sequencing, or WGS, might be done. In WGS, researchers look at all of your genes and at almost all of your DNA. In "standard" genetic testing, researchers look at specific genes or subsets of genes, but not at all genes. Some possible genetic testing is described below.

For each of the questions below, choose the response that matches what you want by putting your initials in the space provided. Please ask the staff any questions that you have before you indicate your selections.

<u>Research Without Human Genetic Testing – OPTIONAL (Research on leftover blood; no human genetic testing)</u>

If you agree, some of your blood that is left over after all required study testing is done may be stored (with usual protection of your identity) and used for ACTG-approved HIV-related research that does not include human genetic testing.

____ (initials) I understand and I agree to this storage and possible use of my blood.

OR

____ (initials) I understand but I do not agree to this storage or possible use of my blood.

<u>Research With Human Genetic Testing – OPTIONAL (Human genetic research on leftover blood)</u>

If you agree, some of your blood that is left over after all required study testing is done may be stored (with usual protection of your identity) and used for ACTG-approved HIV-related research that includes human genetic testing, and may include whole genome sequencing (WGS).

____ (initials) I understand and I agree to this storage and possible use of my blood.

OR

____ (initials) I understand but I do not agree to this storage or possible use of my blood.

<u>Sharing Genetic Data - OPTIONAL</u>

Genetic Research Databases: If you agreed to possible genetic testing of your blood above, researchers may want to share genetic information (with protection of your identity) with other researchers around the world, so that they can learn more about the causes and treatment of diseases. They may store this information in dbGaP, a genetic database maintained by the National Institutes of Health, as well as in other protected databases.

____ (initials) I understand and I agree to this possible sharing of my genetic data.

OR

____ (initials) I understand but I do not agree to this possible sharing of my genetic data.

WHAT IF I HAVE TO STOP TAKING BOTH STUDY-PROVIDED AND NON-STUDY-PROVIDED ART?

During the study:
If you must stop taking ART for 7 or more days in a row before the week 24 study visit and you have not had an HIV-1 RNA test showing that your viral load was <50 copies/mL, then you will be asked to complete the discontinuation evaluations before having to stop the study medication and be taken off the study. The study staff will discuss other options that may be available to you.

If you must stop taking ART for 7 or more days in a row **either during the first 24 weeks after you have had HIV-1 viral load <50 copies/mL or after week 24 on Parts 1 and 2 of the study, or if you have two HIV-1 viral loads in a row that are >200 copies/mL at week 24 or after on Part 1**, you will be asked by the study staff to return to the clinic to have a repeat HIV-1 viral load test that will check whether your ART regimen is working. This test is called a virologic failure (VF) confirmatory test. If the confirmatory test results show that your ART regimen is working for you, then you will remain in the study for continued follow-up and no more PBMC and plasma collection **and no optional procedures** will be done after week 48 **on Part 1 of the study**. If the test results show that your ART regimen has failed, then you will be asked to complete the discontinuation evaluations before having to stop the study medication and being taken off the study. The study staff will discuss other options that may be available to you.

After the study:
After you have completed the study, the study will not provide you with study drugs. The study staff will talk with you about your choices. You and your doctor will decide what treatment you should have, and the study staff will discuss with you how you may be able to obtain ART after the study ends.

WHAT ARE THE RISKS OF THE STUDY?

The main risks of the procedures and study-provided drug are described below.

Risks of Social Harm
Although the study site will make every effort to protect your privacy and confidentiality, it is possible that other people could find out that you are in a study and this could cause problems for you. For example, other people might figure out that you are infected with HIV-1. If this happens, you could be treated unfairly or you could have problems being accepted by other family members, friends, and/or the community.

Risks of Drawing Blood
Taking blood may cause some discomfort, lightheadedness, bleeding, swelling, or bruising where the needle enters the body, and in rare cases, fainting or infection.

Risks Related to Pregnancy
The ART in this study may be unsafe for unborn babies. If you are having sex that could lead to pregnancy, you must agree not to become pregnant or to attempt to make a woman pregnant.

Because of the risk involved, you and your partner must use at least one effective method of birth control. You must continue to use birth control while receiving study drugs.

Remember: If you are having sex, you need to use condoms to prevent transmitting your HIV-1 infection to others.

Approved methods of birth control are listed below. The study staff will talk with you about your choices.

- Birth control medications that prevent pregnancy given as pills, shots, or placed on or under the skin
- Male or female condoms with or without a cream or gel that kills sperm
- Diaphragm or cervical cap with a cream or gel that kills sperm
- Intrauterine device

If you can become pregnant, you must have a pregnancy test, and the test result must be available at the study clinical research site before you can start ART. If you think you may be pregnant at any time during the study, you must tell the study staff right away. If you become pregnant during the study, you may choose to stay in the study [*Sites: Please modify this language as necessary to comply with local/national guidelines*]. The study staff will talk to you about your choices.

If you become pregnant while on study, the study staff would like to obtain information from you about the outcome of the pregnancy (even if it is after your participation in the study ends). If you are taking anti-HIV drugs when you become pregnant, your pregnancy will be reported to an international database that collects information about pregnancies in women taking anti-HIV drugs. This report will not use your name or other information that could be used to identify you.

Risks of ART

All ART medications can have side effects. The drug regimen provided in this study may have side effects. Listed below are the more serious or common side effects that may be related to the study-provided drugs. Please note that these lists do not include all the side effects seen with the study-provided drugs. The staff will be able to tell you which are the most serious side effects. They will also be able to tell you what to do if you have any of these side effects. If you have questions concerning the additional study-provided drug side effects, please ask the medical staff at your site.

BIC/FTC/TAF

BIC/FTC/TAF (50/200/25 mg) is a fixed dose combination tablet containing three medications: bictegravir (BIC, B, GS-9883), emtricitabine (FTC, F), and tenofovir alafenamide (TAF). The safety information known about this tablet is from studies GS-US-380-1489 and GS-US-380-1490, in which 634 patients who had never been treated for HIV received B/F/TAF (50/200/25 mg) for 96 weeks. Adverse drug reactions that have been identified are as follows:

- **Very common: headache, diarrhea, nausea.**
- **Common: vomiting, abdominal pain, indigestion, passing gas, fatigue, and rash.**

- **Other adverse reactions: hives (urticaria) and swelling of the face, lips, tongue, or throat (angioedema).**

If you are living with hepatitis B virus (HBV), there is a possibility of an unexpected worsening of hepatitis B if you stop taking BIC/FTC/TAF.

In a study in pregnant rats, a possible effect on the fertility of male and female baby rats born to mothers given BIC 300 mg/kg/day was observed. The decreases in fertility were slight and remained within the range seen in animals not given any drug in other studies. At the next lowest dose, 10 mg/kg/day, no effects were noted in the baby rats. At this dose, the mother's BIC plasma exposure was approximately eight times higher than the estimated blood concentrations of BIC in humans when administered as BIC/FTC/TAF (50/200/25 mg).

Please talk to your study doctor for more details on adverse events or see the BIC/FTC/TAF package insert for more information.

Emtricitabine (FTC)
The following side effects have been associated with the use of FTC:
- Headache
- Dizziness
- Tiredness
- Inability to sleep, unusual dreams
- Loose or watery stools
- Nausea or vomiting
- Abdominal pain
- Rash, itching, which sometimes can be a sign of an allergic reaction
- Darkening of the skin on the palms of the hands and/or soles of the feet
- Increased cough
- Runny nose
- Abnormal liver function tests, which could mean liver damage
- Increases in pancreatic enzyme (a substance in the blood), which could mean a problem with the pancreas
- Increased triglycerides (a type of fat found in the blood)
- Increased creatine phosphokinase (a substance found in the blood), which could mean muscle damage

NOTE: If you are infected with both hepatitis B and HIV-1, your liver function tests may increase and symptoms caused by hepatitis may get worse if you stop FTC.

Lactic acidosis (elevated lactic acid levels in the blood) and severe hepatomegaly (enlarged liver) with steatosis (fatty liver) that may result in liver failure, other complications, or death have been reported with the use of antiretroviral nucleoside analogues, such as FTC, when used alone or in combination. The liver complications and death have been seen more often in women on these drug regimens. Some nonspecific symptoms that might indicate lactic acidosis

include: unexplained weight loss, stomach discomfort, nausea, vomiting, fatigue, cramps, muscle pain, weakness, dizziness, and shortness of breath.

Some side effects of FTC may not need any medical attention. As your body gets used to the medicine, these side effects may disappear.

Tenofovir Alafenamide (TAF)
The following side effects have been associated with the use of TAF:
- Nausea, vomiting, gas, loose or watery stools
- Generalized weakness
- Dizziness
- Depression
- Headache
- Abdominal pain
- Worsening or new kidney damage or failure
- Inflammation or swelling and possible damage to the pancreas and liver
- Shortness of breath
- Rash
- Allergic reaction: symptoms may include fever, rash, nausea, vomiting, loose or watery stools, abdominal pain, achiness, shortness of breath, a general feeling of illness, or a potentially serious swelling of the face, lips, and/or tongue
- Bone pain and bone changes such as thinning and softening, which may increase the risk of breakage
- Muscle pain and muscle weakness
- Sleeping problems

NOTES:
- If you are infected with both hepatitis B and HIV-1, your liver function tests may increase and symptoms caused by hepatitis may get worse if you stop TAF.
- Because there is only a small amount of information on TAF in pregnant and breastfeeding women, you should not use TAF during pregnancy or if breastfeeding.

Lactic acidosis (elevated lactic acid levels in the blood) and severe hepatomegaly (enlarged liver) with steatosis (fatty liver) that may result in liver failure, other complications, or death have been reported with the use of antiretroviral nucleoside analogues, such as FTC, when used alone or in combination. The liver complications and death have been seen more often in women on these drug regimens. Some nonspecific symptoms that might indicate lactic acidosis include: unexplained weight loss, stomach discomfort, nausea, vomiting, fatigue, cramps, muscle pain, weakness, dizziness, and shortness of breath.

Some side effects of TAF may not need any medical attention. As your body gets used to the medicine, these side effects may disappear.

Cobicistat (COBI)
The following side effects have been associated with the use of COBI:
- Abdominal or stomach pain

- Bloody urine
- Chills
- Clay-colored stools
- Dark urine
- Decreased frequency or amount of urine
- Dizziness
- Fast heartbeat
- Fever
- Headache
- Hives or welts, itching, or rash
- Hoarseness
- Increased thirst
- Irrita**bility**
- Joint pain, stiffness, or swelling
- Loss of appetite
- Lower back or side pain
- Nausea and vomiting
- Pain in the groin or genitals
- Redness of the skin
- Sharp back pain just below the ribs
- Swelling of the eyelids, face, lips, hands, lower legs, or feet
- Tightness in the chest
- Troubled breathing or swallowing
- Unpleasant breath odor
- Unusual tiredness or weakness
- Vomiting of blood
- Weight gain
- Yellow eyes or skin
- Dark-colored urine
- Muscle cramps or spasms
- Muscle pain or stiffness
- Diarrhea
- Discouragement
- Feeling sad or empty
- Irritability
- Loss of interest or pleasure
- Trouble concentrating
- Trouble sleeping
- Upper abdominal or stomach pain

Some side effects of COBI may not need medical attention. As your body gets used to the medicine, these side effects may disappear.

Elvitegravir (EVG)
The following side effects have been associated with the use of EVG:
- Diarrhea
- Headache
- Nausea
- Discouragement
- Feeling sad or empty
- Heartburn
- Indigestion
- Irritability
- Lack of appetite
- Loss of interest or pleasure
- Rash
- Stomach discomfort, upset, or pain
- Thoughts or attempts at killing oneself
- Trouble concentrating
- Trouble sleeping
- Unusual tiredness or weakness
- Vomiting

Some unwanted effects may be caused by EVG. In the event that any of these side effects do occur, they may require medical attention. Some of the side effects that can occur with EVG may not need medical attention. As your body adjusts to the medicine during treatment, these side effects may go away. Your health care professional may also be able to tell you about ways to reduce or prevent some of these side effects. If any of the aforementioned side effects continue, are bothersome, or if you have any questions about them, check with your health care professional.

Some side effects of EVG may not need any medical attention. As your body gets used to the medicine, these side effects may disappear.

Use of Combination Antiretroviral (ARV) Drugs
In some people with advanced HIV-1 infection, symptoms from other infections or certain diseases may occur soon after starting combination ART but can also occur later. Some of these symptoms may be life threatening. If you start having new symptoms, or notice that existing symptoms are getting worse after starting your ART, tell your health care provider right away.

The use of potent ARV drug combinations may be associated with an abnormal placement of body fat and wasting. Some of the body changes include:
- Increase in fat around the waist and stomach area
- Increase in fat on the back of the neck
- Thinning of the face, legs, and arms
- Breast enlargement

There is a risk of serious and/or life-threatening side effects when non-study-provided medications are taken with the study-provided drugs. For your safety, you must tell the study doctor or nurse about all medications you are taking before you start the study. It is also important that you do not start any new medications while on the study before discussing it with the study doctor or nurse. Also, you must tell the study doctor or nurse before enrolling in any other clinical trials while on this study.

CAN I JOIN THE STUDY IF I AM CURRENTLY PREGNANT OR BREASTFEEDING?

If you are pregnant or breastfeeding, you are allowed to take part in the study as long as you meet the requirements of the study. If you are pregnant at the time of enrollment, you will be instructed by the study staff to take alternative, non-study-provided ART during your pregnancy or while breastfeeding.

WHAT IF I BECOME PREGNANT DURING THIS STUDY?

If you become pregnant while on study, you must tell the study staff right away and see your doctor to get the best care possible. You will be asked to return to the clinic for a study visit at your earliest possible convenience. You may remain on study during your pregnancy or you may leave the study. Either way, the study staff will contact you to find out about any events that happen during your pregnancy and about the health of the baby.

If you choose to stay in this study, you will not be able to continue on the study-provided drug. You and your study doctor will decide what ART are best for you now that you are pregnant. If you are on study-provided EVG/COBI/FTC/TAF **or BIC/FTC/TAF**, you will need to change to alternative non-study-provided ART that is felt to be appropriate for use in pregnancy by your study doctor and/or primary care provider. You will continue to have regularly scheduled study visits, but you will not take part in the optional procedures and large volume blood collection. This study will not provide care related to your pregnancy, the delivery of your baby, or the care of your baby. You must arrange for your care and your baby's care outside of this study. Your study doctor will help you find appropriate care.

Long-term follow-up is recommended for a baby whose mother takes ART during pregnancy. The study staff will talk with you about long-term follow-up and the possibility of enrolling your baby in a long-term follow-up study.

After your baby is delivered, you may be able to return to the study-provided drug regimen you were taking before you became pregnant. **It is unknown if the study-provided drugs pass through the breast milk and whether this may produce adverse effects in your baby. It is also unknown if taking the study-provided drugs will reduce the risk of passing HIV to your baby while breast feeding.** If you decide to breastfeed your baby, you **cannot remain on the study-provided drug regimen and** must **continue taking** your current non-study-provided ART **prescribed by your doctor**.

WHY WOULD THE DOCTOR TAKE ME OFF THIS STUDY EARLY?

The study doctor may need to take you off the study early without your permission if:

- You never started ART (study-provided or non-study-provided drugs) or started ART later than 48 hours after joining the study.
- An HIV-1 test shows that you are not infected with HIV or you have had HIV infection for a longer period of time than expected or your laboratory tests from entry show that you do not qualify for the study.
- You miss 3 study visits in a row.
- Your viral loads show that the HIV medications you are taking are no longer working well for you **or if you have two viral loads in a row that are >200 copies/mL at week 24 or after on Part 1**; this is known as having VF.
- **You stop taking ART for 7 days or more in a row, and you have not had a viral load <50 copies/mL (before stopping ART that lasted ≥7 days in a row) during Part 1.**
- Your doctor believes that remaining on the study is no longer what is best for you.
- You are unable to follow the requirements of the study.
- **The study is stopped or cancelled.**

The study doctor may also need to take you off the study-provided drugs without your permission if:

- Continuing the study-provided drugs may be harmful to you, for example, if the study drugs are making you sick.
- You need a treatment that you may not take while on the study-provided drugs.
- You become pregnant and/or start breastfeeding.

If you must stop taking ART before the study is over or if you want to stop the study visits, then the study staff will ask you to return to the clinic for a final visit.

If you are taken off the study early because you never started ART, you do not need to have a final visit.

WHAT ARE THE BENEFITS OF BEING IN THIS STUDY?

It is possible that being in this study will be of no direct benefit to you and that the optional procedures being done will not change your clinical care.

It is also possible that being in this study benefits you in one of the following ways:

- Gives you access to ART (for about 1 year).
- Gives you more detailed information about your HIV-1 infection than you could get from your local anti-HIV care center; having this information could help you get better treatment for your HIV-1 infection.
- Provides you with frequent health checks that could help identify problems early; having this information could help you get good treatment at the right time.

Finally, it is possible that your being in this study will provide information that will help others with HIV-1 infection.

WHAT OTHER CHOICES DO I HAVE BESIDES THIS STUDY?

Instead of being in this study, you have the choice of:
- treatment with the anti-HIV drugs available to you locally
- treatment with experimental drugs, if there is a study available locally for which you qualify
- no treatment

Please talk to your doctor about these and other choices available to you. Your doctor will explain the risks and benefits of these choices.

[Sites may insert general information about HIV/AIDS or other local study-specific treatment availability.]

WHAT ABOUT CONFIDENTIALITY?

For Sites in the US
We will do everything we can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, we have a Certificate of Confidentiality from the US Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. Also, any publication of this study will not use your name or identify you personally.

Your records may be reviewed by the ACTG, **the US** Office for Human Research Protections (OHRP), **or other local, US, and international regulatory entities** as part of their duties (insert name of site) institutional review board (IRB) (a committee that protects the rights and safety of participants in research), National Institutes of Health (NIH), study staff, study monitors, drug companies supporting this study, and their designees. Having a Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we **are** required to tell the proper authorities.

A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by US law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

For Sites outside the US
Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Any publication of this study will not use your name or identify you personally.

Your records may be reviewed by the ACTG, the US Office for Human Research Protections (OHRP), **or other local, US, and international regulatory entities** as part of their duties (insert name of site) institutional review board (IRB) or Ethics Committee (a committee that protects the rights and safety of participants in research), **National Institutes of Health (NIH), study staff, study monitors, drug companies supporting this study,** and their designees.

A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by US law. This website will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this website at any time.

WHAT ARE THE COSTS TO ME?

You will not have to pay for the study-provided drug, EVG/COBI/FTC/TAF **or BIC/FTC/TAF**. If your doctor decides that other drugs would work better for you, then you might have to pay for those drugs. This could happen if you are unable or unwilling to take EVG/COBI/FTC/TAF **or BIC/FTC/TAF** or if you become pregnant while on study.

You will not have to pay for study visits, exams, and laboratory tests needed for this study. The study will perform one resistance test for all participants (at entry prior to taking the study medications) and possibly another resistance test in those participants whose ART regimen has failed (at the VF confirmatory visit). In addition, some of your blood may be tested at the end of the study to look at genetic factors. The results of these tests will not be available right away since the tests will be done later in the study.

Taking part in this study may lead to added costs to you or your insurance company. In some cases, it is possible that your insurance company or health care system will not pay for these costs because you are taking part in a research study. *[Sites: Delete references to insurance company or health care system if not applicable at site.]*

WILL I RECEIVE ANY PAYMENT?

You will be reimbursed for the study visits and for the optional procedures done between weeks 60 and 72 (such as gut biopsy and lumbar puncture). *[Sites: If the participants will receive payment, describe the amount to be paid, the payment schedule and any prorated schedule should the subject decide to withdraw or is withdrawn early by the investigator.*]

WHAT HAPPENS IF I AM INJURED?

If you are injured as a result of being in this study, you will be given immediate treatment for your injuries. *[Sites: Please indicate whether this treatment will be provided free of charge, or whether the participant must pay the costs].* There is no program for compensation either through this institution or the NIH. You will not be giving up any of your legal rights by signing this consent form.

WHAT ARE MY RIGHTS AS A RESEARCH PARTICIPANT?

Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. Your decision will not have any impact on your participation in other studies conducted by NIH and will not result in any penalty or loss of benefits to which you are otherwise entitled. You will still be able to receive drugs to treat your HIV-1 infection outside this study.

We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. If you want the results of the study, let the study staff know.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study or a research-related injury, contact:
- name of the investigator or other study staff
- telephone number of above

For questions about your rights as a research participant, contact:
- name or title of person on the IRB or EC or other organization appropriate for the site. An IRB or EC is a group of people not connected with this study who will monitor the study at this site.
- telephone number of above

SIGNATURE PAGE: A5354 INFORMED CONSENT

If you have read this consent form (or had it explained to you), all your questions have been answered and you agree to take part in this study, please sign your name below.

Participant's Name (print)	Participant's Signature and Date
Study Staff Conducting Consent Discussion (print)	Study Staff's Signature and Date
Witness's Name (print) (As appropriate)	Witness's Signature and Date

ATTACHMENT A: Study Visits

The study staff can answer any questions you have about individual study visits, the evaluations that will occur, or how long each visit will be. The table below can be used as a quick reference for you, along with the explanations that follow.

I. Study Schedule **for Part 1**

Evaluation or Procedure	Screening[1]	Entry[2]	Most Other Visits[3]	Some Other Visits[4]	Special Visit[5]	Leaving or Stopping the Study Early[6]
Consent	✓			✓		
Acute HIV-1 infection documented	✓					
Medication history	✓	✓				
Medical history	✓	✓				
HIV-1 infection confirmed and Fiebig stage testing		✓				
Group assigned and ART started		✓				
Physical exam		✓	✓			✓
ART modifications			✓			✓
Blood collected		✓	✓		✓	✓
Pregnancy test		✓	**If suspected**			✓
Resistance test		✓			✓	
Urine collected		✓				✓
Telephone follow-up with participants				✓		
Adherence support			✓	✓	✓	
Large volume blood draw		✓				
Consent and Perform Optional procedures				✓		
Approximate amount of blood		up to 359 mL	see footnote 3 below for blood volume details	see footnote 4 below for blood volume details	20 mL	9 mL

II. Study Schedule for Part 2

Evaluation or Procedure	Step 2 Entry[2]	Every 24 weeks until week 144[3]	Special Visit[5]	Leaving or Stopping the Study Early[6]
Physical exam	✓	✓		✓
ART continued	✓	✓		
ART modifications	✓	✓	✓	✓
Blood collected	✓	✓	✓	✓
Pregnancy test	✓	**If suspected**		✓
Adherence support	✓	✓	✓	
Approximate amount of blood	**up to 83 mL**	**up to 83 mL/visit**	**14 mL**	**19 mL**

[1]Screening: After you have read and signed the consent form, the study staff will check your medical records for available documentation of your HIV-1 diagnosis; for instance, whether you are recently or acutely infected with HIV-1 to make sure that you meet the requirement for joining the study. **You will be asked about anti-HIV and other kinds of medications you have taken in the past.**

[2]Entry Visit: If you are eligible to join the study, you will enter **Part 1 of** the study and be placed into a group. At **the entry** visit **on Part 1 of the study**, you will start taking ART. If you are unable or unwilling to take the study-provided drug, then you must have access to and can begin to take an alternative ART in order to enter the study.

NOTE: **In Part 1 of the study,** large volume blood collection (**about 320** mL of blood) will be done, and at some sites, **optional** leukapheresis may be done in place of large volume blood collection at entry.

If you remain eligible after completing Part 1 of the study, you will enter Part 2 of the study. You will continue to take ART while on Part 2. If you are unable or unwilling to continue taking the study-provided drug, then you must have access to and can begin to taking an alternative ART in order to remain on the study.

[3]Most Other Visits: Most participants will be seen at 1 week, 4 weeks, 12 weeks, and 24 weeks after entering the study and then at 48 weeks and thereafter at weeks 60 and 72. Those participants whose test results show Fiebig VI will have their final study visit at week 24 **on Part 1 of the study**. **Participants in Part 2 of the study will be seen every 24 weeks until week 144.**

The **approximate** amount of blood (in mL) that will be drawn at study visits **will be from 3 mL (or about 1 tsp) to 173 mL (or about 35 tsp).**

[4]Some Other Visits: At weeks 2 and 8 **(of Part 1)** and the day after having the optional procedures (gut biopsy and/or lumbar puncture), you will be contacted by the study staff over the phone to check how you are doing. At week 36, you will come to the clinic to have a physical exam, a viral load test, and an adherence evaluation.

At weeks 60 and 72, you will have blood drawn (23 mL or about 5 tsp) for routine safety labs at each visit. Depending on whether or not you reached the main goal of the study at week 48, you may be asked as to whether you are willing to have additional **optional** procedures done and samples collected between weeks 60 and 72 (see Appendix II for details on these procedures).

[5]Special Visit: **You may be asked to return for an extra visit at week 49 if you need to have blood drawn for an HIV test and other research tests.** If it seems that the ART regimen is not working or if you are having side effects from your treatment regimen, then you may be asked to come to the clinic for an extra visit.

[6]Leaving the Study Early: You will be asked to come to the clinic for an extra visit if you leave the study or stop the study treatment early.

III. Explanation of Evaluations

Consent and contact information collection
After you read the consent and have had a chance to ask questions about the study, you will sign the consent form if you want to continue to be evaluated for study participation. You will also be asked how to be contacted in case you miss a visit or there are problems with your tests, and whether you give the study team permission to contact you.

Acute HIV-1 infection documentation
The study staff will check your medical records to see if you had an HIV test done as part of local routine care and whether you were recently or acutely infected with HIV-1.

HIV-1 infection confirmation and Fiebig staging
Blood will be collected to confirm that you have HIV-1 infection, and an additional blood sample will be collected for Fiebig staging (a test to show how far along you are with your HIV-1 infection).

Group assignment and study treatment/locally-provided ART
You and the clinic staff will discuss your assigned group and the optimal ART options for you. These will be based on your acute HIV-1 diagnosis and medical history. At study entry, you will receive either study-provided or other non-study-provided ART based upon your discussion with the study site doctor. The study staff and the study pharmacist will tell you how often to take your drugs and whether you should take them with food.

Physical examination
You will have a physical exam and will be asked questions about your health and about any medicines you have taken or are taking now. **You will also be asked about any symptoms and illness you may have had since the last visit.**

ART modifications
You will be asked if there has been any change in how you take your study-provided or non-study-provided anti-HIV medications since the last visit.

ART continued
In Step 2, you will be asked to confirm that you continue to take your study-provided or non-study-provided anti-HIV medications since the last visit.

Blood collection
Blood will be collected from you for various tests during the study. These include: routine safety laboratory tests, HIV-1 viral load (a test that shows how much HIV-1 is in your blood), CD4+ T-cell count (a test that shows how many infection-fighting cells you have in your blood), and liver function tests.

NOTE: For Part 2 of the study, laboratory tests, CD4+ T-cell count, and liver function tests may be collected from clinical records.

At entry and at the confirmatory visit to check whether your ART regimen has failed, a sample will be collected for resistance testing (a test that shows whether the ART is working for you). Routine PBMC and plasma storage will be done.

Human genetic testing
Some of your blood will be tested to see whether the ART you are taking are making a difference by looking at your immune response (levels of infection fighting cells, CD4+ and CD8+ T-cells, in your blood) or whether development of resistance to ART is associated with different genes. You will not receive the results of these studies because they will be done in the future.

Pregnancy testing
If you are a woman who is able to become pregnant, then you may be asked to give a small urine or blood sample for a pregnancy test.

HIV-1 resistance testing
Your blood will be used to see which ART might work best for you.

NOTE: If resistance testing was done as part of routine care, the study doctor may review these results to make sure that your current ART is still the best for you.

Urine collection
You will be asked to provide a small amount of urine that will be used in safety tests.

Primary endpoint determination
At week 48, you will be asked to give blood samples for testing to determine whether you met the main goal of the study by looking at the total amount of HIV genetic material in infection-fighting cells at study week 48 (also known as the primary endpoint).

Site follow-up with participants via telephone
You will be asked about how you have been feeling and how well you are remembering to take your ART.

Adherence support
Everyone will get some adherence support from the site staff. This means that the study staff will explain to you in detail how to take the medications and help you find ways to take the medications correctly.

ATTACHMENT B: SAMPLES COLLECTION AND OPTIONAL PROCEDURE
AT **STEP 1** ENTRY

A. Explanation of ***Samples Collection and Optional Procedure***

The samples collection and optional procedure below will advance the scientific goals of this study but will offer no direct benefit to participants. Neither you nor your doctor will receive any results from the samples collection and optional procedure because these tests are for research purposes only. If you choose not to participate in the optional procedure, it will not affect your ability to take part in this study.

NOTE: If you are pregnant, the samples collection and optional procedure will not be done.

- Large volume blood collection

  A large volume blood draw (**about 320** mL) will be collected.

  NOTE: At some sites, leukapheresis may be done in place of large volume blood collection.

- Leukapheresis (in place of large volume blood draw)

  The leukapheresis procedure may be performed at *[insert site-specific details]*. The procedure will take about *[insert site-specific details]* and the full visit will last about *[insert site-specific details]*. You will have to remain in a semi-reclining or reclining position for most of this time.

  Leukapheresis is a medical procedure that involves removing whole blood from an individual/donor and separating the blood into individual components so that leukocytes (white blood cells) can be removed. The remaining blood components are then put back into the bloodstream of the individual/donor. This will be done by inserting a needle attached to sterile tubing in one arm, and first sending your blood through a machine. This machine spins your blood to separate the red blood cells (cells that carry oxygen), the white blood cells (cells that fight infection) and the platelets (cells that help form clots). The white blood cells will be kept for testing. The rest of your blood will be returned to your body through another needle and tube in your other arm. Not all of your white blood cells are removed, and your body will make more white cells within a few days. Losing the number of white blood cells that are collected does not pose a danger to your health.

B. Risks Associated with ***Samples Collection and Optional Procedure***

- Large volume blood collection

  Taking blood may cause some discomfort, lightheadedness, bleeding, swelling, or bruising where the needle enters the body, and in rare cases, fainting or infection.

- Leukapheresis (in place of large volume blood draw)

  Leukapheresis has been shown to be safe in donors **living with HIV** and does not affect CD4+ T-cell count or immune status of short-term donors. The needle used is larger than normal blood draw and may be uncomfortable. Rarely, a participant may feel faint during or after leukapheresis. This sort of reaction can be handled by changing the participant's position or administering intravenous fluids. You may experience chills, nausea, and heartburn caused by the citrate anticoagulant that is used during the procedure to keep the collected cells from clumping together in the bag. This chemical may use up some of the calcium in your blood stream, and tingling in the face, lips, or hands may be noted. If this happens, study staff may slow the rate of infusion of this chemical and may offer you one or two calcium carbonate tablets to correct the calcium loss. Participants will be observed closely by an experienced blood bank technician during the procedure.

APPENDIX II: CONSENT FOR OPTIONAL PROCEDURES AND SAMPLES COLLECTION AFTER WEEK 48 ON STEP 1

If you met the main goal of the study (i.e., to look at the total amount of HIV genetic material in infection fighting cells at week 48), you may be asked and consented to take part in the optional procedures and samples collection that will be done between weeks 60 and 72. The optional procedures and samples collection (as described below) will advance the scientific goals of this study but will offer no direct benefit to participants. Neither you nor your doctor will receive any results from these procedures because these tests are for research purposes only. If you choose not to participate in the optional procedures, it will not affect your ability to take part in this study.

NOTES:

1. **About 25 people participating in this study will take part in these optional procedures.**
2. If you are pregnant, these optional procedures and samples collection will not be done. **If you stop taking ART for 7 days or more in a row or if you have a viral load that is confirmed to be more than or equal to 50 copies/mL, you will not be able to participate in these optional procedures.**

A. Explanation of Optional Procedures and Samples Collection

- Gut biopsy

  The gut biopsy procedure will be performed at *[insert site-specific details]*. The procedure will take about *[insert site-specific details]*, and the full visit should last about *[insert site-specific details]*.

  A gut biopsy is a medical procedure that involves removing a sample of tissue taken from your gut to closely examine it. This will be performed following the local standards of care for this procedure. Just before the gut biopsy, you may have an enema (a salt water rinse that will flush out your lower bowel). Next, a lubricated flexible tube will be placed into your rectum. Using this instrument, the doctor will examine the inside of your intestine and will collect samples of tissue for testing.

  You should not have anal sexual intercourse for 3 days before and for 7 days after the procedure. The study or clinic staff will call you the day after the procedure to check on how you are feeling.

  [Note to sites: Insert details regarding how this procedure is performed in your institution and whether an additional consent form is required at the clinic where it will be performed].

- Lumbar puncture

  A lumbar puncture is a medical procedure that involves removing a small amount of cerebrospinal fluid (CSF) from your spine. You should drink plenty of fluids the day

before the lumbar puncture procedure. The procedure will be performed at *[insert site-specific details]*. You will be asked to lie down on your side or to sit "backwards" in a chair (so that you are facing the back of the chair). An area of skin on your lower back will be sterilized with fluid. You will get an injection to numb the skin in the sterilized area. You may feel a burning sensation from the fluid that is injected. When the area is numb, the doctor will insert a thin needle between two of the bones in your spine. A small amount of fluid will be collected through the needle. The entire lumbar puncture procedure to this point will take about 30 minutes.

After the CSF collection, you may be asked to lie flat for up to 30 minutes to reduce the chance that you will get a headache. You should limit your physical activity for the remainder of the day. The study or clinic staff will call you the day after the procedure to check on how you are feeling.

B. Risks Associated with Optional Procedures and Samples Collection

- Gut biopsy

  The preparation before the procedure may include taking laxatives or enema, which may be uncomfortable and may make participants feel weak from diarrhea. You may feel pressure or discomfort as the instrument (a lubricated flexible tube called a sigmoidoscope) is placed into your rectum as it passes through the curves of your colon during the procedure. You may receive medication throughout the procedure to reduce any discomfort. The doctor will put air into your colon in order to see the lining, and you may have some bloating or abdominal discomfort from the air. You may feel as though you need to have a bowel movement. You can pass the air if you feel the need. The doctor will remove as much air as possible after the procedure.

  There is a small risk (about 1/3,600) for bowel perforation which is a tear through the wall of the bowel that may allow leakage of bowel fluids. Perforations are generally treated with hospitalization, antibiotics, and possibly surgery. Bleeding can occur but usually will stop without treatment or can be controlled at the time of the procedure. Rarely, blood transfusions or other treatments may be required to stop the bleeding.

  After the procedure, you may feel dizzy or sleepy from the sleep and pain medicines. You should have someone come with you to accompany you home. You should not drive a car or a motorcycle. There is a small risk (less than 1/10,000) for a more severe allergic reaction to the sleeping medicine.

- Lumbar puncture

  The risks of lumbar puncture include local soreness at the site of needle entry and pain and possible allergic reaction associated with local anesthesia. There is a small risk of headache or decreased blood pressure from removing the small amount of fluid or leaking of CSF after the procedure. There is a small risk of infection and a very small risk of damage to nerves in the lumbar spinal roots after the procedure, which could cause pain, numbness, or loss of sensation to the legs. Before the procedure, the area where

the needle will be inserted will be cleaned with antiseptics (such as betadine or rubbing alcohol) in order to reduce the risk of infection. A bandage will be placed on the skin where the needle went in, and the participants will be asked to remove it the next day and tell the study doctor right away if any redness or tenderness is present. Participants will be asked to remain lying flat for up to 30 minutes after the procedure and will be given fluid to drink after the procedure. The site staff will ask the participants about history of any allergies to anesthetics and will not perform lumbar puncture in any participant with such history.

Please indicate below if you agree to have any of these **optional** procedures and large volume blood collection done. No matter what you decide, it will not affect your participation in the study. You will not receive the results of these studies because they will be done in the future. You do not have to decide about the optional procedures and blood collection until week 48 of the study.

Gut biopsy

_____ (initials) YES, I agree _____ (initials) NO, I do not agree

Lumbar puncture

_____ (initials) YES, I agree _____ (initials) NO, I do not agree

SIGNATURE PAGE: A5354 INFORMED CONSENT FOR OPTIONAL PROCEDURES

If you have read this consent form (or had it explained to you), all your questions have been answered, and you agree to take part in the optional procedures that will be done between weeks 60 and 72 **on Part 1 of the study**, please sign your name below.

Participant's Name (print)	Participant's Signature and Date
Study Staff Conducting Consent Discussion (print)	Study Staff's Signature and Date
Witness's Name (print) (As appropriate)	Witness's Signature and Date