Modulating the Impact of Critical Events in Early HIV Infection: Effect of ART Initiation and Alcohol use

The MERLIN Study

Sponsored by:

National Institute on Drug Abuse (NIDA)
U. S. National Institutes of Health (NIH)

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Version 3.0
April 12, 2018

FHCRC IRB Approval
FEB 11 2021
Document Released Date

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SIGNATURE PAGE

Modulating the Impact of Critical Events in Early HIV Infection: Effect of ART Initiation and Alcohol use

Sponsored by:

National Institute on Drug Abuse (NIDA)
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I, the Principal Investigator, agree to conduct this study in full accordance with the provisions of this protocol. I will comply with all requirements regarding the obligations of investigators. I also agree to maintain all study documentation for at least two years.

I have read and understand the information in the Investigator's Brochures and/or package inserts, including the potential risks and side effects of the products under investigation, and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

Ann Duerr, MD, PhD, MPH
Principal Investigator and Protocol Chair

_____ _____
Signature of Principal Investigator Date

EXECUTIVE SUMMARY

Protocol Title: Modulating the Impact of Critical Events in Early HIV Infection: Effect of ART Initiation and Alcohol use

Short Protocol Title: The MERLIN Study

Sites: IMPACTA San Miguel and Barranco Clinical Research Sites at IMPACTA PERU Clinical Trials Unit in Lima, Peru.

Planned Starting Date: January 2017

Planned Ending Date: December 2022

Study Rationale: This study will investigate the relative long-term benefit of early initiation of antiretroviral therapy (ART) in men who have sex with men (MSM) and transgender women (TW) with recently-acquired HIV infection. The impact of early ART initiation will be assessed in participants according to their Fiebig stage at diagnosis. Fiebig staging uses the presence of HIV RNA, p24 and patterns of antibodies bands seen on western blots to define 5 stages during the first 3-4 months after HIV acquisition plus a 6^{th} stage for long-term infection.

The overall objective is to determine the impact of time of ART initiation and high-level alcohol use on a number of HIV-related clinical parameters. These include time to viral suppression, CD4 count, HIV reservoir, changes in the gut microbiome and neurocognitive functioning.

Primary Objectives:

1) To determine the relative long-term benefits of immediate vs. early vs. delayed (by 6 months) initiation of ART.

2) To determine the impact of high-level alcohol use on the relative long-term benefits of immediate vs. early vs. delayed ART initiation

Primary Endpoints (1):

1) Measure the impact of early treatment on cell-activation, and inflammation by comparing cellular markers of inflammation in study participants who initiated treatment at varying times after HIV acquisition.

2) Measure the impact of early treatment on the replication-competent HIV reservoir (size of the reservoir and types of cells involved) in study participants who initiated treatment at varying times after HIV acquisition.

3) Determine whether early ART initiation prevents long-term changes in gastrointestinal tract bacterial microbiota and correlate these changes with systemic markers of inflammation and disease progression such as the activity of the kynurenine pathway of tryptophan catabolism and plasma concentrations of the inflammatory cytokine interleukin-6 (IL-6).

4) Document the impact of early ART initiation on 'non-HIV-associated' diseases such as neurocognitive disorders. Assess the correlation of these clinical outcomes with laboratory markers of cell activation, inflammation and HIV reservoir size and characteristics.

5) Determine the impact of high-level alcohol use on the outcomes listed in 1-4, after controlling for time of ART initiation.

Primary Endpoints (2):

1) Examine the time course of seeding of the replication-competent HIV reservoir quantifying the size of the reservoir and identifying the types of cells involved at time-points close to HIV acquisition.

2) Characterize innate immune responses (cytokines and other soluble mediators) shortly after infection and determine the impact of these responses on the strength and character of the subsequent adaptive immune response to HIV, as well as the size and characteristics of the HIV reservoir (see above).

3) Probe changes in gastrointestinal tract bacterial microbiota shortly after HIV infection and determine the correlation of these changes with two established markers of disease progression: the activity of the kynurenine pathway of tryptophan catabolism and plasma concentrations of the inflammatory cytokine interleukin-6 (IL-6).

Design: The MERLIN Study extends the follow-up of participants enrolled in the SABES Study, a study of ~250 MSM and TW in Lima with acute and recent HIV infection enrolled in an early vs. deferred antiretroviral treatment study. The MERLIN study will offer follow-up to all of the patients enrolled in the SABES study, prioritizing the participants who also were enrolled in a related Observational Study, in which baseline peripheral blood mononuclear cell (PBMC) isolation sampling was conducted. Additionally to the participants previously enrolled in SABES, up to 25 additional participants with acute HIV diagnosis will be enrolled directly in this study and will be treated immediately upon diagnosis.

Consenting eighteen-year old or older MSM and TW, who participated and received ART in the *SABES* Study and who have acceptable hematologic, liver, and renal function will be considered to participate in the MERLIN study, in which they will continue a 3-drug ART regimen.

Because of the low number of participants, which acute infection who received immediate treatment in the SABES study and who could be eligible for MERLIN, a new enrollment and follow-up cohort will be open with up to 25 participants (see above). In this cohort, participants with acute HIV infection will initiate antiretroviral treatment immediately after receiving diagnosis and will also be followed up for an extended time.

Duration: Up to 5 years from enrollment on this protocol (through 2022) with visits timed referencing date of HIV diagnosis and original Sabes study visits (or SABES enrollment if applicable.

Treatment Regimen:

In *SABES* Step 3, participants had been randomized to either one of the following arms: Immediate Arm: to immediately receive ART for 48 weeks; or Deferred Arm: to delay treatment for 24 weeks (unless Peruvian criteria for initiation of treatment are met), and then receive ART at 24 weeks.

For the purposes of correlative studies, participants in the Immediate Arm are further categorized as initiating Immediate ART (in Acute HIV: Fiebig I/II) or Early ART (Recent HIV: Fiebig III/V) infection.

The MERLIN participants will initiate the co-formulation EVG/COBI/FTC/TDF (Genvoya) for its component TAF.

EVG/COBI/FTC/TDF is not readily available in private or public sector HIV care in Peru, but it is considered acceptable based on FDA-approval and US-based HIV guidelines to the Peruvian Ministry of Health (MOH).

Participants receiving protease-inhibitor (PI)-based regimens at MERLIN enrollment will be transitioned to Genvoya after individual evaluation.

- **Group A:** This group will be comprised of persons who initiated EFV/FTC/TDF during *SABES* Step 3.

  These participants will continue with EVG/COBI/FTC/TAF.

- **Group B:** This group will be comprised of persons who initiated EFV/FTC/TDF during *SABES* Step 3 and completed all 48 weeks of the protocol before MERLIN (or the Observational Study) began enrollment.

  These participants were transferred to continue their in the MOH/TARGA (Peruvian Government) HIV clinics and received non-study first-line ART (EFV + lamivudine (3TC) + zidovudine (AZT), or other regimen) after completing *SABES* Step 3 and up to their enrollment in the MERLIN study.

  These participants will continue treatment with EVG/COBI/FTC/TAF.

- **Group C**: This group will be comprised of persons who initiated EVG/COBI/FTC/TDF during *SABES* Step 3 or other persons currently taking a PI or INSTI-based regimens. Because INSTI-based regimens are considered preferential as described above, at MERLIN enrollment, these participants will be transferred to EVG/COBI/FTC/TAF. Persons who require to take PI-based regimens have generally had EFV intolerance/hypersensitivity or even virologic resistance. For these reasons, they cannot receive other treatments. All persons receiving PI-based regimens will also be transitioned to EVG/COBI/FTC/TAF unless a clinical contraindication exists.

- **Group D**: This group will consist of participants who were diagnosed with acute infection

and enrolled in MERLIN immediately after their diagnosis and who will initiate antiretroviral treatment immediately with EVF/COV/FTC/TAF.

Evaluations: All participants will be evaluated at regular intervals for primary and secondary outcomes.

BACKGROUND

4.1 INTRODUCTION

Recent data suggest that initiation of antiretroviral therapy very early after HIV acquisition can confer several benefits. These include reduction in the size of the HIV reservoir and decrease in HIV-associated systemic inflammation that has been linked to non-AIDS mortality in HIV-infected participants, even when HIV replication is effectively controlled by antiretroviral therapy (ART). In addition, some patients who are treated very early appear to effectively control HIV replication years later when ART is discontinued, sometimes referred to as a functional cure. However, very early initiation of ART (in Fiebig stages I or II [FI or FII] prior to seroconversion) does not prevent the establishment of HIV reservoirs, which eventually expand in most patients when ART is discontinued [1, 2]. Early ART initiation is, nonetheless, associated with preservation of CD4+ T cells, preserving normal gut microbiota, averting a major driver of HIV-related immune activation, and limiting the size of the HIV reservoir [3]. When, by contrast, ART is initiated after seroconversion (in Fiebig stage III [FIII] or later), many HIV-associated changes have already occurred, including seeding of HIV reservoirs, damage to GI mucosa, and initiation of inflammatory cascades [3]. Some if not all of these effects may be related to the development of dysbiosis (a markedly different composition of the gut microbiota that might itself drive continued inflammation).

Since ART initiation within weeks of HIV acquisition is not a viable public health strategy, it is important to more completely understand the *relative long-term benefits* of initiating ART at very early times after infection (FI-II) as opposed to after a short (during FIII-V) or longer delay (at 24 weeks). Because most HIV-infected individuals come to medical attention long after they have been infected, data on individuals with known infection dates, especially those who were infected very recently, is a valuable resource. The MERLIN study of HIV-infected (HIV+) participants will allow the collection and analysis of clinical data and biologic specimens at multiple time points from HIV+ participants with known infection dates in Lima. It will focus on studies of HIV clinical course which incorporate data on time of infection, duration of infection prior to initiation of antiretroviral therapy (ART), and molecular markers of infection such as inflammation and immune dysfunction (including innate immune responses), size and location of HIV reservoirs, and changes in the microbiome. In addition, it will follow participants clinically, with collection of data such as CD4 count, neurocognitive function etc. The effects of alcohol on microbial translocation and pro-inflammatory cytokines are not unlike early changes after HIV infection [4-6]; however, alcohol's role in HIV pathogenesis and progression is not well defined. Since the possibility that high-level alcohol use can mitigate the benefits of early ART is emerging as a potentially important public health issue, the MERLIN study will evaluate the impact of alcohol use as well as time of ART initiation on HIV pathogenesis in Peruvian MSM and TGW in whom high-level alcohol use is common.

The overarching hypotheses of the MERLIN study are as follows: 1. Initiation of ART soon after HIV infection will dampen perturbations of GI microbiota, reduce HIV-induced inflammatory

changes, and decrease seeding of the reservoir. Initiation of ART in FI-II will have the greatest benefit. CD4 counts and peripheral inflammatory markers in the FIII-V group and the group treated at 24 weeks will approach those in the FI-II group at 1.5 and 3.5 years after ART initiation; in contrast, changes in the GI microbiome and the HIV reservoir over time will be more modest. 2. Irrespective of the time of ART initiation, alcohol use will compound the negative effects of HIV to generate greater levels of dysbiosis, microbial translocation, up-regulation of inflammatory pathways, and seeding of the HIV reservoir.

Specimens from the *SABES* and MERLIN studies will be used for a series of laboratory investigations probing the mechanisms by which early treatment reduces the HIV reservoir and HIV-associated inflammation, and seeking to determine how soon after HIV acquisition ART must be initiated to confer these beneficial effects. The most in-depth studies will prioritize participants enrolled during the latter half of the *SABES* study who had large PMBC collections at the time of HIV diagnosis and throughout follow-up. These include the following 3 groups based on time of ART initiation: a) *Immediate*: during FI-II (N~20), b) *Early*: during FIII-V (N~35) or c) *Deferred*: at 24 weeks after diagnosis (N~55). A small number of additional studies may be conducted on all participants in the *SABES* study: a) *Immediate*: during FI-II (N~35), b) *Early*: during FIII-V (N~72) or c) *Deferred*: at 24 weeks after diagnosis (N~115). The MERLIN study will assess outcomes after 1.5 and 3.5 years after initiation of ART in these MSM and TGW. The results will be evaluated every 6 months during the follow-up of the participants in the study (up to the end of 2022). Laboratory analyses will be conducted in Lima, Perú and Seattle, WA as well as in the laboratories of our collaborators at University of California, San Francisco (UCSF) and University of Montreal (UoM). Additional neuropsychiatric and biologic data will be collected to evaluate neuro-inflammation and cognitive changes in early HIV infection (collaborators at UCSF, Yale, and UNC).

4.2 JUSTIFICATION

Early ART initiation is associated with preservation of CD4+ T cells, reduced immune activation (lower levels of $CD4^+$ and $CD8^+$ T cell activation and lower soluble inflammatory markers), lower total HIV DNA levels, and lower cell-associated RNA levels [7]. Recent studies suggest that very early initiation of ART (prior to seroconversion in humans or before detection of viremia in non-human primates) does not prevent the establishment of HIV reservoirs, which eventually expand when ART is discontinued [2, 8]. However, early initiation of ART prevents loss of gut Th17 cells thereby reducing HIV-related immune activation and limits the size of the HIV reservoir [1, 3]. Drug and/or alcohol use in North and South American MSM is associated with increased risk of acquiring and transmitting HIV, and we hypothesize it will influence the course of HIV infection as well. In Peru, where we have enrolled a cohort of MSM/TGW with acute or recent HIV infection, alcohol use is common. In a recent study, 52% of Peruvian MSM reported alcohol use during sex while only 5% reported concomitant drug use. MSM from the US participating in the same study reported drug and alcohol use of 39% and 38%, respectively [9]. A 2011 survey of 5,148 gay and bisexual men in 5 Peruvian cities reported alcohol use disorder (AUD), defined as a score ≥8 on the Alcohol Use Disorder Identification Test (AUDIT), in 63% of respondents [10, 11], five times higher than that reported by males in the general Peruvian population [12].

4.2.1 Timing of ART Initiation and Progression of HIV Disease

Even in the setting of suppressive ART, persistent elevations in T cell activation and systemic inflammation appear to play a central role in HIV disease progression. Early ART initiation is associated with lower levels of $CD4^+$ and $CD8^+$ T cell activation, lower total HIV DNA levels, and lower cell-associated RNA levels in subjects with virologic suppression [7]. In a small number of individuals who were treated very early and then stopped ART, viral suppression continues off therapy [13-15]. Six months of ART initiated shortly after seroconversion (during FIII) improved many immune parameters, although not to the level seen when ART is initiated prior to seroconversion (during FI-II) which resulted in near normal (HIV-uninfected) levels for some parameters [3]. These results raise the question of how to translate these findings to a clinical setting. Recent studies suggest that initiation of ART during hyper-acute infection (i.e., Fiebig stages I and II) may be optimal, but detecting such individuals and linking them rapidly to care pose substantial logistical challenges.

4.2.2 Microbial Translocation, GI Microbiome, and Inflammation in HIV Disease

The GI mucosa is involved in HIV-induced inflammation directly through microbial translocation, which results in increased levels of circulating LPS [16], and indirectly through up-regulation of inflammatory responses in dendritic cells in response to IFN-γ and HIV Tat and Nef. This in turn has detrimental effects on Th17 cells in the gut mucosa [17, 18], leading to impairment of mucosal integrity. Alterations in the gastrointestinal (GI) microbiome, called dysbiosis, also contribute to these inflammatory processes [19, 20]. Dysbiotic bacteria enriched in HIV infection produce tryptophan catabolites (e.g., of the kynurenine pathway) which are linked to inflammation and have been associated with translocation of microbial products into the systemic circulation [21, 22]. Thus, the bacterial communities in HIV-infected individuals may be important drivers of the inflammatory processes that contribute to disease. Importantly, effective ART does not completely reverse the immunopathologic changes in GI mucosa in all subjects, raising the possibility that differences among ART-treated subjects may be related to whether ART was initiated prior to ‘irreversible’ establishment of dysbiotic GI microbial communities.

4.2.3 Alcohol Effects on Microbial Translocation and the GI Microbiome

Importantly for this protocol, alcohol use has also been linked to microbial translocation and increased levels of endotoxins, such as lipopolysaccharide (LPS), in the portal and systemic circulation. Binge drinking in healthy individuals with no history of AUD results in significant, rapid, and transient increases in serum LPS and 16S rDNA, as well as lipoprotein binding protein(LBP) and soluble CD14 (sCD14) [23], likely due to microbial translocation from the gut to the circulation. Gut microbiome shifts in AUD include reductions in the normally dominant species and enrichment of Gammaproteobacteria and depletion of Bacteroidetes [4], two taxa that are similarly altered in the gut of HIV-infected subjects and linked with HIV disease progression [19, 21, 22]. Thus, alcohol use may exacerbate microbial translocation, inflammation and HIV disease progression by inducing qualitative and/or quantitative changes in these microbial communities.

4.2.4 Alcohol Use, Systemic Inflammation, and Immunity

Innate immune responses appear to initiate the inflammatory cascade linked to alcohol use in animals and in humans [24]. Chronic alcohol consumption has been linked to increases in

circulating levels of LPS, the pro-inflammatory cytokines [5], and acute-phase proteins [6]. These changes are likely mediated at least in part by microbial translocation: thus, microbial pathogen-associated molecular patterns interact with host receptors and trigger downstream transcription and expression of inflammatory genes. Alcohol may also interfere with granulocyte and monocyte production [25], impair the function of dendritic and phagocytic cells [26] and decrease natural killer (NK) cell activity [27].

4.2.5 Effect of Alcohol on HIV Progression is Unclear

There is substantial overlap between the effects of alcohol and HIV on microbial translocation, changes in GI flora, and systemic inflammation. Indeed, some *in vitro* and animal studies link alcohol use to higher plasma HIV/SIV RNA viral loads (VL) [28] and accelerated progression of SIV disease [29], although this finding has not been replicated in all studies [30]. Human studies of alcohol and HIV infection report mixed results; some small studies suggest poorer outcomes [31, 32] while larger cohort studies found no effect on VL or $CD4^+$ T cell counts in ART-naïve or treated patients [33]. The differences may be due to unmeasured confounders, poor ART adherence among treated patients, and/or inaccurate assessment of alcohol use (e.g., due to single or widely-spaced measurements, social desirability bias, and use of non-standard definitions). Regardless, the aforementioned detrimental effects of alcohol on microbiome composition, microbial translocation, and systemic inflammation suggest that it may exacerbate HIV disease, and that treatment of AUD in the context of HIV infection may help to resolve inflammation and diminish the viral reservoir.

4.2.6 HIV Integration Sites

Recent studies of individuals who initiated ART during chronic infection [34, 35] indicate that HIV integration into genes associated with cancer or cell cycle regulation confers a survival advantage to specific cells, apparently the result of increased proliferation and formation of clonal populations that persist during suppressive ART. Identifying which early integration events lead to proliferation and persistence of infected cells may suggest novel interventions to reduce or clear the HIV reservoir that is limited in size due to early ART. We expect that participants who initiate ART early vs. later after primary infection will have fewer unique integration sites prior to initiation of ART and at 18 and 42 months will have fewer proviruses in genes associated with cancer and cell proliferation. Also, we expect that AUD will correlate with T cell activation and inflammatory markers. Therefore, we hypothesize that individuals with low alcohol use will have relatively fewer unique integration sites at all time-points compared to those with high use who initiate ART at the same Fiebig stage, and at 18 and 42 months will have fewer proviruses in genes associated with cancer and cell proliferation.

4.2.7 Neurocognitive Impairment

HIV-associated neurocognitive disorders (HAND) are caused by the impact of HIV infection on the central nervous system (CNS). HIV infection of the CNS is associated with neuronal damage, inflammation and, in approximately 40% of treated individuals, cognitive impairment [36-38].Both CNS pathology and cognitive impairment can persist despite antiretroviral therapy (ART) when it is initiated during established HIV infection; and cognitive impairment may associate with persistent HIV replication and HIV-driven inflammation in the CNS. One intriguing possibility is that CNS HIV persistence may be reduced by very early initiation of ART. In collaborative studies

of individuals treated immediately with acute HIV infection in Thailand [41, 42], we have shown that key markers of inflammation in the CNS quickly normalize and that cognitive function remains normal over the first 6 months of longitudinal follow-up[43]. Among HIV-infected individuals in the US, we found that those initiating ART earlier in the first year of HIV infection (i.e., during "recent" HIV infection) had significantly better cognitive function after a year of ART than did those in whom treatment was more delayed. These observational data strongly suggest—but do not prove—that there is some window in the first year of infection (i.e., during acute and/or recent infection) during which ART can keep HIV from developing the ability to replicate efficiently and persist locally in the CNS on ART. Both alcohol and stimulant use can augment systemic inflammation, increase local neuroinflammation and cause direct neuronal injury. Alcohol-induced neurotoxicity overlaps with HIV mechanisms of neuropathogenesis through oxidative stress, overproduction of pro-inflammatory factors, impairment of blood–brain barrier and glutamate associated neurotoxicity [44]. Thus, it is possible that higher psychostimulant and alcohol use can lead to accelerated or less reversible neurologic and neurocognitive effects.

Collectively, these results suggest that alcohol may compound the negative effects of HIV to generate greater levels of dysbiosis, microbial translocation, up-regulation of inflammatory pathways and seeding of the HIV reservoir. They also suggest that initiation of ART soon after HIV infection might: 1) dampen perturbations of GI microbiota, 2) reduce HIV-induced inflammatory changes, and 3) decrease seeding of the reservoir, although to a lesser degree in those with AUD. We will explore these questions in MSM/TGW (N~240) with acute or recent HIV infection, a large proportion of whom report high-level alcohol use. The proposed research will be conducted in MERLIN, as an extension of our ongoing study of acute and recent HIV infection among MSM/TGW in Peru, entitled the '*SABES*' study. (https://clinicaltrials.gov/ct2/show/NCT01815580?term=*SABES*&rank=1).

4.3 PROBLEM UNDER INVESTIGATION

Results of several recent studies suggest that initiation of ART soon after HIV infection can reduce HIV-induced inflammatory changes and seeding of the reservoir. Clinical studies also suggest that early initiation of therapy can have a beneficial impact by reducing the HIV-associated morbidity that persists even when HIV replication is successfully achieved through ART. These studies leave open the question of how soon after HIV acquisition ART must be initiated to achieve these beneficial results. While initiation of ART during hyper-acute (Fiebig stages I and II) might seem optimal, detecting such individuals and linking them rapidly to care poses huge logistical challenges, not the least of which is instituting more wide spread screening for HIV infection using NAAT (nucleic acid amplification test). The intent of this study is to determine the impact of early treatment initiation among patients with acute (HIV RNA+/ seronegative) or very recent (HIV seropositive with a documented HIV RNA tested within the past 3 months) HIV infection. Most participants in this study participated in the *SABES* study (see below) and initiated ART early after HIV acquisition. Because participants were randomized to start ART at different times, the proposed studies will allow assessment of the relative impact of ART initiated at various times during the first year of infection. In addition, a small number of participants with chronic HIV infection and a small number of HIV-uninfected participants will be enrolled for comparison.

5. HYPOTHESIS

ART initiation during FI-II stages will largely prevent changes in GI microbiota and inflammatory markers, and reduce the size of the reservoir of replication-competent HIV.

1) Over time CD4 counts and peripheral inflammatory markers in seropositive participants who initiate ART 2 to 8 months after infection (ie in the Early and Deferred Arms) will approach those who initiated ART during Acute (FI-II) HIV; in contrast, we expect that these individuals initiating ART subsequent to acute infection will demonstrate persistent changes in the GI microbiome and in the HIV reservoir.

2) High-level alcohol use will compound the negative effects of HIV to generate greater levels of dysbiosis, microbial translocation, and up-regulation of inflammatory pathways, leading to increased seeding of the HIV reservoir prior to ART initiation. Early treatment should diminish, but not completely prevent, the changes in the microbiome associated with alcohol consumption and their impact on immune activation and seeding of the HIV reservoir.

6. OBJECTIVES

The overall objective is to determine the impact of time of ART initiation and high-level alcohol use on a number of HIV-related clinical parameters. These include time to viral suppression, CD4 count, HIV reservoir, changes in the gut microbiome and neurocognitive functioning.

6.1 PRIMARY OBJECTIVES

1) To determine the relative long-term benefits of immediate vs. early vs. deferred initiation of ART.

   Initiation of ART during Fiebig I-II, while theoretically appealing, is currently infeasible in clinical practice because the duration of FI-II is very short (10 days) and because antibody-based tests, used almost world-wide, are unable to diagnose FI-II. Six months of ART initiated shortly after seroconversion (FIII) can improve many immune parameters, although not to the near normal level seen when ART is initiated during FI-II [3]. Longer-term follow-up of seropositive participants who started ART ~2-8 months after HIV acquisition compared to those initiating ART during FI-II is needed to see whether markers of inflammation and residual viral load might continue to improve after 6 months, decreasing the difference between the groups. ***Methods:*** We will evaluate the 3 groups using specimens from equivalent times after ART initiation through the first 5 years of ART usage, comparing CD4 counts, time to undetectable VL, GI microbiota, and inflammatory markers. The size of the replication-competent HIV reservoir in PBMCs will be compared using measurements of multiply spliced viral RNA (msRNA) and the degree of low-level infection of new cells by assessing the production of circular viral DNA (2LTR HIV DNA). We will model the size and persistence of the viral reservoir across all ART-adherent study participants. In a subset of individuals from each group, the proviral integration sites will

be defined at ART initiation and after 1.5 and 3.5 years to assess the maintenance of the reservoir by proliferating CD4 cells. We will continue evaluating endpoints annually or biannually, as long as participants remain on study.

2) To determine the impact of high-level alcohol use on the relative long-term benefits of immediate vs. early vs. delayed ART initiation

Dysbiosis is present in individuals with high-level alcohol use [23, 24, 27, 45-47] and in many HIV-infected patients. The extent of dysbiosis correlates with established markers of HIV disease progression and the consequent inflammatory processes contribute to HIV disease progression, even in treated individuals. ***Methods**:* We will use monthly self-report data on alcohol use as well as alcohol biomarkers in blood and hair to assess alcohol consumption as a continuous, time-varying predictor. In all participants at the time of diagnosis, and at multiple time points prior to ART in those enrolled into the deferred group, we will assess VL, GI microbiota, pro-inflammatory cytokines, and HIV reservoirs. We will compare these factors between subjects by their reported alcohol use, focusing on events occurring shortly after HIV acquisition. Specimens from uninfected and chronically infected subjects are available as controls. Finally, among ART-adherent subjects with persistent viral suppression, we will HIV DNA integration sites in those with and without high-level alcohol use at 1.5 and 3.5 years after ART initiation.

6.2 SECONDARY OBJECTIVES

1) To estimate the impact of time of initiation of antiretroviral therapy on short- and long-term clinical and laboratory outcomes.

2) To evaluate the correlation between gastrointestinal dysbiosis and systemic immune activation in MSM with HIV and alcohol use disorders.

3) To evaluate the association with alcohol abuse and timing on ART initiation neurocognitive function and neuro inflammation in MSM with acute and recent HIV.

4) To determine whether NNRTI and INSTI-based ART regimens have differential effects on time to HIV VL suppression and size of the latent HIV reservoir.

5) To determine whether NNRTI and INSTI-based ART have differential moderation of immune activation associated with HIV and alcohol abuse.

7. STUDY DESIGN

7.1 GENERAL STUDY DESIGN

This protocol for the MERLIN study describes the extended follow-up of participants enrolled in the *SABES* study, a study of ~250 HIV+ MSM and TGW in Lima, Perú. In this study, we will enroll up to 25 individuals with acute HIV infection.
The *SABES* study: The ongoing *SABES* study combines laboratory and epidemiologic data with

modeling to investigate the hypothesis that early ART initiation and management of alcohol use disorder will prevent onward HIV transmission among MSM/TGW diagnosed with acute (FI-FII: Ab-/HIV RNA+) or recent (≤3 months) HIV infection (FIII-V). The *SABES* study population is MSM and transgender women (TGW) not using feminizing hormones (all referred to as "MSM" from here on) in Lima. This study is being conducted in 3 phases: 1) HIV screening, 2) monthly follow-up testing of HIV-uninfected participants by point-of-care EIA and NAAT testing for HIV RNA in pools of seronegative specimens (pools are run daily), and 3) enrollment of MSM with incident HIV infection into a randomized ART treatment study (**Figure 1**). Only participants with acute (seronegative but HIV RNA+) or recent HIV infection (EIA+ with a prior HIV-negative test within 3 months) are eligible for enrollment in the treatment phase of the *SABES* study. These participants are stratified by acute vs. recent infection and randomized 1:1 to Immediate arm (to receive ART immediately) or Deferred arm (to receive ART beginning at 24 weeks or when the participant meets Peruvian treatment initiation thresholds, whichever comes first) (**Figure 1**). We completed enrollment of 225 HIV+ participants into the *SABES* study by the end of March 2016. A small number (5-10) of additional participants will be enrolled in *SABES*; those with symptomatic acute HIV infection will receive ART immediately, in accordance with current Peruvian treatment guidelines and will therefore not be randomized to possibly delay ART. The Human Subjects section contains a discussion of risks and benefits. All *SABES* participants received either EFV/FTC/TDF or EVG/COBI/FTC/TDF as first line medication. Virologic suppression at 48 and 96 weeks is equivalent for both regimens when used as initial treatment [48]; however because EVG/COBI/FTC/TDF may result in more rapid viral suppression we began using EVG/COBI/FTC/TDF for new participants when it became available as a donated drug in late 2015.

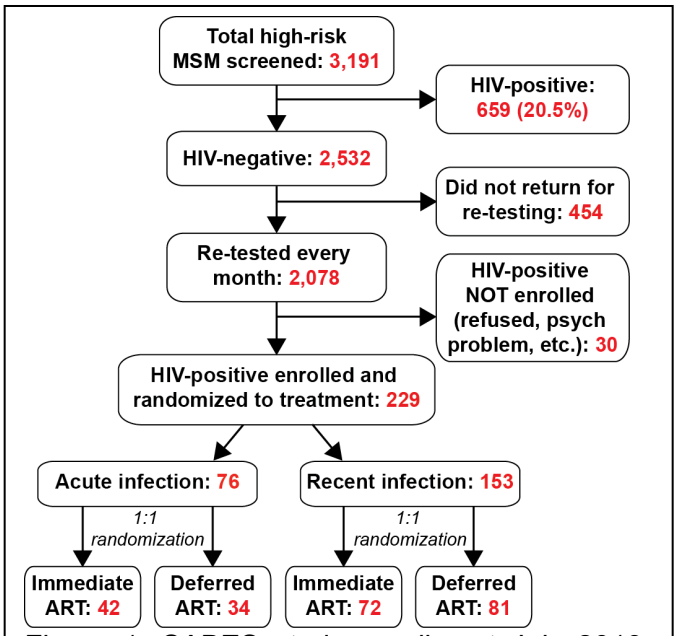

Figure 1: SABES study enrollment July 2013-July 2016

Specimen collection: The MERLIN study involves laboratory analysis of specimens collected during the 48-week *SABES* study as well as during the MERLIN study, through 2022 (about 5 years after MERLIN enrollment) . Visits with specimen collection occurred in the *SABES* study at 1, 2 and 4 weeks after ART initiation (study weeks 1, 2, 4 for the Immediate arm and weeks 25, 26, 28 for the Deferred arm); additional visits occur at 8, 16, 24, 32, 40 and 48 weeks. Extensive samples, including blood, stool for microbiome, and dried blood spots and hair for drug and alcohol tests were collected at each study visit for *SABES* study participants, starting at participant number 110. In addition, during the latter half of the *SABES* study, neurocognitive testing was conducted starting at participant 110, as well as collection of cerebrospinal fluid (CSF) by lumbar punctures on a subset to investigate the early neurologic impact of HIV infection. For the MERLIN study, we will collect blood, urine, rectal secretions and rectal biopsies, and stool specimens every 24 weeks throughout 2022, as well as additional neurocognitive testing and collection of CSF on a subset of participants.

7.2 MANAGEMENT OF SUBJECTS WITH VIROLOGIC FAILURE OR PREMATURE DISCONTINUATION OF TREATMENT

Virologic failure is defined if there is an HIV-1 RNA of >400 copies/mL in 2consecutive measurements after having reached an HIV-1RNA level of <50copies/mL in 2 consecutive measurements separated by at least 14 days.

If there is a sub-optimal virologic response, the Virologic Laboratory of the University of Washington will perform genotyping from a sample taken from before discontinuation of the treatment when the HIV-1 RNA is >500 copies/ML with the goal of determining if there has been development of resistance or not, and if yes, to help in the selection of a new antiretroviral regimen. The substituted regimen will be provided by the study while the participant is participating in the study. If the genotype does not reveal evidence of resistance and there are convincing arguments (that is to say, adherence has improved substantially or there were intercurrent illnesses that could explain the elevated viral load) the participant could continue with the original treatment plan. Any decision to continue with the regimen in the context of a suboptimal viralogic response must be discussed with the PI/co-PI of the study. The subsequent management of whether or not to initiate a new regimen or modify the regimen must be at the discretion of the local investigator in concordance with the standard of care. Subjects that remain in the study will be requested to complete all of the described evaluations during which time they will be provided an alternative antiretroviral treatment provided by the study.

8. SELECTION AND ENROLLMENT OF SUBJECTS

8.1 DESCRIPTION OF THE POPULATION

Consenting MSM and TW eighteen-year old or older, who participated and received ART in the *SABES* Study; acceptable hematologic, liver, and renal function to support an approved 3-drug ART regimen.

8.1.1 Inclusion Criteria

1) Age ≥18 years

2) MSM or TW

3) Able to give consent, including consent for medical procedures, blood draws, and switching antiretroviral medication if indicated

4) Participants with acute/recent HIV infection who have participated in *SABES*

    a. Current *SABES* participants, receiving ART in their assigned treatment by the time this study is implemented; or

    b. *SABES* participants who completed 48 weeks of study participation in their

assigned treatment arm and were rolled over into the ART program sponsored by the Peruvian MOH;

c. Any participants of the Collaborative Observational Protocol who do not meet criteria for a or b above

5) Acute/Recent HIV Participants: Already on ART, without treatment interruption, either:

a. Receiving co-formulated EFV 600 mg, FTC 200 mg, and TDF 300 mg; or

b. Receiving co-formulated EVG 150 mg, COBI 150 mg, FTC 200 mg, and TDF 300 mg (Stribild); or

c. Receiving a combined ART regimen sponsored by the Peruvian MOH

d. Prior/current participation in *SABES* and now receiving a non-EFV or EVG regimen with 3 active drugs (3 drugs not inclusive of the boosting agents COBI or ritonavir)

6) Seronegative participants with acute HIV infection (lack of antibodies against HIV and presence of HIV-1 RNA.

7) Kidney, liver, and hematologic functions should not prohibit ongoing administration of study drugs or else another clinically-appropriate 3-drug ART regimen (3 active drugs not inclusive of boosting agents ritonavir or COBI)

8) HIV Viral load <1000 copies/ml on the last available result (either routine clinical or study result).

9) No medical or psychiatric conditions that render the person a poor study participant in the opinion of the study physician.

10) Co-enrollment with other protocols will be at the investigators discretion.

8.1.2 Exclusion Criteria

1) Chronic hepatitis B co-infection

2) Use of drugs that are highly dependent on CYP3A for clearance (such as rifampin) which preclude administration of one of the study ART regimens or else other clinically-indicated 3-drug ART regimen

3) Concurrent use of aminoglycosides, IV amphotericin B, cidofovir, cisplatin, foscarnet, IV pentamidine, probenecid, adefovir or immunomodulatory agents

4) Any other medical or social condition that in opinion of the Investigator could affect the assessment of study objectives or put volunteers at risk.

8.2 RECRUITMENT

After the completion of the last *SABES* Study visit, participants on ART will be invited to participate in the MERLIN Study. Participants who have already completed follow-up in the *SABES* Study and were retained in clinical follow-up at IMPACTA and other clinics and received ART through a Peruvian MOH program will also be invited to participate. *SABES* participants will be eligible to transfer to the MERLIN Study (either at the end of their 48-week follow-up or when the MERLIN Study opens whichever occurs later).

To complete the initial cohort of the SABES 2017 study, enrollment will be offered to additional participants with acute HIV infection if those were diagnosed while seronegative, at the study site or collaboration institutions, and if they are interested in initiating antiretroviral treatment immediately in this study.

9. STUDY TREATMENT

9.1 TREATMENT REGIMENS

All participants receiving EFV-based will receive EVG/COBI/FTC/TAF.

EVG/COBI/FTC/TAF will be initiated in all participants because of the benefit of TAF compared with TDF and could have an improved metabolic risk and has demonstrable benefit on renal and bone protection

All Group C participants should be transitioned to EVG/COBI/FTC/TAF as soon as drugbecomes available for distribution in the study.

All participants in groups A and B who initiated EVG/COBI/FTC/TDF should transition to EVG/COBI/FTC/TAF as soon as that drug becomes available in the study.

Participants who have initiated EVG/COBI/FTC/TAF could be switched to other antiretroviral treatment only if there is evidence of intolerance.

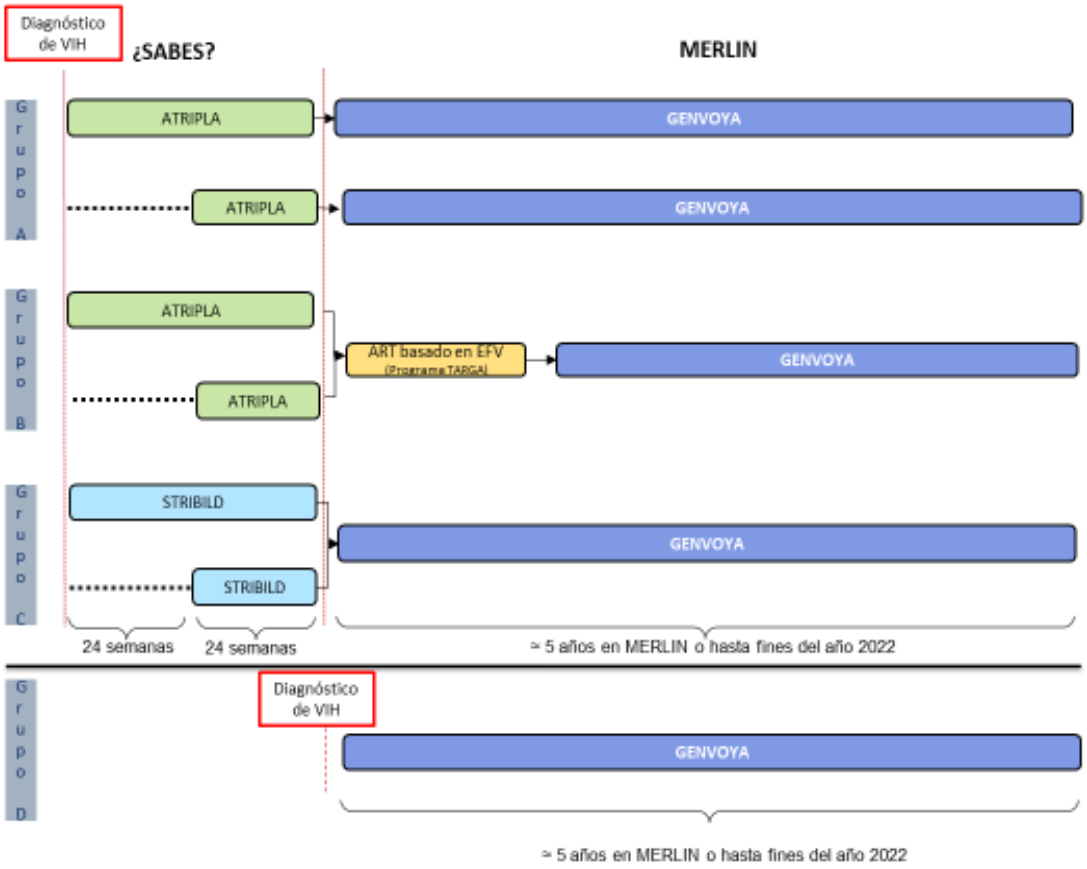
Diagnóstico de VIH
¿SABES?
MERLIN
Grupo A
ATRIPLA
GENVOYA
ATRIPLA
GENVOYA
Grupo B
ATRIPLA
ART basado en EFV
(Programa TARGA)
GENVOYA
ATRIPLA
Grupo C
STRIBILD
GENVOYA
STRIBILD
24 semanas
24 semanas
≈ 5 años en MERLIN o hasta fines del año 2022
Grupo D
Diagnóstico de VIH
GENVOYA
≈ 5 años en MERLIN o hasta fines del año 2022

Figure 2.

9.2 ADMINISTRATION

EVG/COBI/FTC/TAF or EVG/COBI/FTC/TDF will be administered as one tablet orally once daily with food. Other ART regimens will be administered as per respective packet instructions.

9.3 DURATION

Participants will remain in the study through 2022 (about 5 years), regardless of time since HIV diagnosis and/or completion of the *Sabes* protocol. The visit weeks for MERLIN will continue to be counted with respect to HIV diagnosis/Sabes 3 enrollment. ART will be provided by the study for the duration of the time that the participant remains in the study. At the end of study, participants will continue ART from other sources, including transition to Peruvian MOH clinics.

9.4 STUDY PRODUCT FORMULATION AND PREPARATION

Elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (GENVOYA®) is co-formulated to contain 150 mg of elvitegravir, 150 mg of cobicistat, 200 mg of emtricitabine, and 10mg of tenofovir alafenamide in each tablet.

Emtricitabine/tenofovir DF (Truvada®) is co-formulated to contain 200 mg of emtricitabine and 300 mg of tenofovir DF and will be available when will be necessary to change the antiretroviral treatment.

Each bottle of the described medicaments contain a silica gel desiccant that should be kept in its original recipient to protect the product from humidity. The original recipient can be dispensed. Store at 25°C (77°F), variations from 15°-30°C (59°-86°F) are allowed.

9.5 PRODUCT SUPPLY

Co-formulated EVG/COBI/FTC/TDF (STRIBILD®) and EVG/COBI/FTC/TAF (GENVOYA®) will be provided by Gilead Sciences, Inc. Co-formulated emtricitabine/tenofovir DF (Truvada®) will be provided by Gilead Sciences, Inc. Truvada will be used only in combination with at least a third antiretroviral. Per discretion of the investigators, other drugs could be provided procured locally if one of the listed study drugs cannot be used.

9.6 PHARMACY FACILITIES

The pharmacy facilities are located at the IMPACTA Barranco and IMPACTA San Miguel Clinical Research Sites and both are staffed by registered trained pharmacists. Both pharmacies will have adequate space to store sufficient quantities of study agent to assure continuous access to all study participants. The study drug will be stored in accordance with the drug manufacturer's recommendations. Both pharmacies' storage and the study pharmacies will be locked by a secure door. The pharmacies and storage facilities have climate-controlled environments, with controlled humidity and temperature to remain within limits allowed by the manufacturer for drug storage.

9.7 DRUG INVENTORY

The pharmacists at the IMPACTA Barranco and IMPACTA San Miguel Clinical Research Sites will receive the study agent and store it in the pharmacy. Access will be restricted to authorized pharmacy personnel only. The pharmacists will be responsible for keeping accurate records of the material received. At the end of the study, the pharmacists will perform the final drug accounting of unused study material on the proper log documents. Unused study agent will be disposed of in accordance with local regulations.

9.8 DRUG DISPENSING

The Study Pharmacist will be responsible for dispensing the drug to the Study Nurse who will deliver it to the participant. At each visit, the pharmacist will receive the prescription that includes the participant's enrollment ID number.The pharmacist will dispense the drug according to the procedures shown in Figure 2. For bottle dispensed, the pharmacist will enter the packed label information and date in a Drug Dispensation Log, to be kept secure in the Pharmacy.

9.9 CONCOMITANT MEDICATIONS

To avoid adverse events caused by drug interactions, investigators must refer to the most recent package insert for study drug and concomitant agents whenever a concomitant medication is initiated or a dose is changed.

Research sites must also refer to the study product's most recent package insert or investigator brochure to access additional current information on prohibited and precautionary medications.

Below are lists of selected concomitant medications. These lists are only current as of the date of this protocol. Therefore, 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 inserts or investigator brochure to obtain the most current information on drug interactions, contraindications, and precautions.

9.9.1 Precautions with Concomitant Medications

Package inserts of antiretroviral drugs and concomitant agents should be referenced whenever a concomitant medication is initiated or a dose is changed to avoid drug interaction AEs.

9.9.2 Prohibited Medications

Co-administration with drugs that are highly dependent on CYP3A for clearance and for which elevated plasma concentrations are associated with serious and/or life-threatening events is contraindicated. These drugs and other contraindicated drugs (which may lead to reduced efficacy of STRIBILD and possible resistance) are listed in the table below.

Table 1a: Drugs that are Contraindicated with STRIBILD and GENVOYA

MEDICATION CLASS	**PROHIBITED MEDICATIONS**
ALPHA 1-ADRENORECEPTOR ANTAGONIST	Alfuzosin
ANTIMYCOBACTERIAL	Rifampin
ERGOT DERIVATIVES	Dihydroergotamine Ergotamine Methylergonovine
GI MOTILITY AGENT	Cisapride
HERBAL PRODUCTS	St. John's wort (*Hypericum perforatum*)
HMG-COA REDUCTASE INHIBITORS	Lovastatin Simvastatin
NEUROLEPTIC	Pimozide
PHOSPHODIESTERASE-5 (PDE5) INHIBITOR	Sildenafil when dosed as REVATIO for the treatment of pulmonary arterial hypertension
SEDATIVE/HYPNOTICS	Triazolam Orally administered midazolam

* Some brand names are showed, but attention must be taken about the generic name.
** A single dose of midazolam may be used for sedation in subjects undergoing procedures in a monitored setting.

9.9.3 Options for Medication Substitutions Due to Intolerance/Toxicity

If intolerance or toxicity occurs, substitutions may be made to the study regimen, after discussion with the Protocol Chair, as long as the new regimen is consistent with current guidelines for potent ART. The substituted medications will be provided by the study for the duration of time that the participant remains in the study. The purpose of the study is to determine whether ART in general, not necessarily **a** specific regimen, affects the long-term clinical benefit for MSM and TG with different alcohol consumption levels, who acquired HIV recently. However, because in this study we are performing additional analysis comparing treatments based on EFV and EVG, those participants undergoing these medicament substitutions could be excluded from such secondary analyses.

9.9.4 Options for Medication Substitutions for Virologic Failure

If a subject develops virologic failure on the study regimen further management based in clinical judgment should proceed at the discretion of the On-Site Principal Investigator in consultation with the Protocol Chair or Co-Chair.

Substitutions in antiretroviral regimens during the course of the study are at the discretion of the site investigator. It is the site investigator's responsibility to review all concomitant medications to ensure safety. The protocol pharmacist is available for discussion if questions arise.

The substituted medications will be provided by the study for the duration of time that the participant remains in the study.

9.10 ADHERENCE ASSESSMENT

Adherence will be assessed via validated self-report measures, pharmacy refill-based measures, and unexpected increases in HIV plasma viral load. Specifically, the Visual Analogue Scale [49] and the Ability Likert item [50] will be used to collect self-reported adherence over the past 30 days, collected monthly. These measures have demonstrated consistent associations with viral load in other studies [51]. Because self-report has a number of limitations, including a tendency of over-estimate adherence in comparison to electronic drug monitoring measures as well as potentially high demands on other cognitive processes to produce accurate reports [52], we also will monitor pharmacy dispensation records to produce medication possession ratios (days covered by a prescription/days between scripts) in approximately 3-month intervals. Pharmacy-based measures of adherence have consistently been associated with clinical outcomes in previous research [53, 54]. We will not monitor ART blood levels because 1) this is not done as part of routine clinical practice, 2) the tests would only refer to recent adherence rather than adherence over time, and 3) it is rather expensive to conduct these tests.

10. STUDY PROCEDURES

10.1 Visit Program

Procedures and Sample collection		Reference to Arm of ART initiation in SABES 3	ENR *(Enrollment with acute infection diagnosis)*	Study week *+/- 28 days*				ENR [3, 4] (enrollment after SABES participation)	Study week *(counted since enrollment into SABES study –after HIV diagnosis) +/- 8 weeks of window*								
																Annual Visits		Bi-annual visits
				12	24	36	48		72	96	120	144	168	192	216	Every 48 weeks after wk 192 for the immediate arm (I)	Every 48 weeks after wk 216 for the deferred arm (D)	Every 24 weeks after: Wk 192 = I Wk216 = D
Informed Consent		both	X					X										
Clinical Procedures[5]		both	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Questionnaire (CASI)		both	X		X		X	X	X	X	X	X	X	X	X	X	X	X
Pneurophsycologic test		both	X		X		X	X	X	X	X	X	X	X	X	X	X	
Abdominal circunference*		both						X	√	√	√	X			X	X	X	
Stool	New ENR	Immediate	X		X		X		X	X	X	X	X	X	X			
	Collection in the past	both						X	X	X	X	X	X	X	X	X	X	
	No collection in the past	Immediate												X[1]				
		Deferred													X[1]			
Rectal biopsy and secretion (N=60)		Immediate								X		X		X				
		Deferred									X		X		X			
Urine		Both	X		X		X	X	X	X	X	X	X	X	X	X	X	

BLOOD																	
ART safety tests (Biochemistry, liver function and hematology)	both	X		X		X	X	X	X	X	X	X	X	X	X	X	X
CD4/CD8	both	X					X	X	X	X	X	X	X	X	X	X	X
HIV viral load	both	X		X		X	X	X	X	X	X	X	X	X	X	X	X
Lipid panel**	both	X				X	X[2]	√[2]	√[2]	√[2]	X			X	X	X	
Glucose**	both	X		X		X	X[2]	√[2]	√[2]	√[2]	X			X	X	X	
PBMC	both	X		X		X	X	X	X	X	X	X	X	X	X	X	
Plasma	both	X		X		X	X	X	X	X	X	X	X	X	X	X	
Whole blood (RNA LATER)	both						X	X	X	X	X	X	X	X	X	X	

Procedures and sample collection	Reference to Arm of ART initiation in SABES 3	ENR *(Enrollment with acute infection diagnosis)*	Study week *+/- 28 days*				ENR [3, 4] (enrollment after SABES participation)	Study week *(counted since enrollment into SABES study –after HIV diagnosis) +/- 8 weeks of window*							Annual Visits		Bi-annual visits
			12	24	36	48		72	96	120	144	168	192	216	Every 48 weeks after wk 192 for the immediate arm (I)	Every 48 weeks after wk 216 for the deferred arm (D)	Every 24 weeks after: Wk 192 = I Wk216 = D
Leukapheresis (N=40)	Inmediato								X		X		X		X		
	Diferido							X		X		X		X		X	
Dried blood	ambos						X	X	X	X	X	X	X	X	X	X	
Syphilis tests	ambos						X	X	X	X	X	X	X	X	X	X	
Max volumen of blood (ml) Including leukapheresis (if applicable)	ambos	250		250		250	250	250	250	250	250	250	250	250	250	250	30

√ conditiona procedure X obligatory procedure
* The measurement must be taken the same day of simple collection for lipid panel and glucose
** fasting

[1] for those in which stool sample was not collected in the Sabes 3 enrollment visit, the sample must be taken in week 192 for the immediate arm participants and in week 216 for the deferred arm participants.

[2] the sample for lipid panel and glucose must be taken during fasting; however, if the participant is not fasting during the enrollment visit, the simple can be recovered in the immediately following visit.

[3] If the enrollment visit occurs within 8 weeks after the Observational study "Collaborative Studies of the Effect of Very Early Initiation of ART and Development of HIV Incidence Assays in Lima, Peru") visit, it will not be necessary to collect samples collected during such visit (PBMC, stool, urine); the only sample to be collected will be for safety test, CD4/CD8, viral load, lipid panel and glucose.

[4] If the enrollment visit occurs within the window period of the week visits, 72, 96, 120, 144, 168, 192, 216 or the following every 6 month visits; then it will not be necessary to collect samples already obtained in the enrollment visit (PBMC, stool, urine) in the next follow-up visit; only a sample to run safety tests should be collected.

[5] Medical chart evaluation, physical exam, concomitant medication and diagnosis will be performed.

[6] For participants in the Group D and any other who did not have lumbar puncture before 2018, the lumbar puncture procedure will not be done in weeks 96/120, but only after week 144 (based on study arm).

[7] The last lumbar puncture procedure will be done at week 240 for the immediate arm and week 264 for the deferred arm.

10.2 RECRUITMENT

10.2.1 SABES 3 Participants

SABES 3 Participants will be recruited from current SABES study participants at any time during SABES 3 follow-up, immediately after the week 48 visit, or after they have entered follow-up care at IMPACTA or TARGA Clinical sites.

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10.2.2 New participants with acute infection

Up to 25 new participants with acute hiv infection will be enrolled. The diagnosis will be done during the acute phase of HIV infection (positive HIV RNA test or p24 antigen with negative HIV antibody test) at the IMPACTA site or other collaboration institutions. These participants will rapidly be referred to this study.

10.3 TIMING OF VISITS

10.3.1 Enrollment Assessments

10.3.1.1 Participants from SABES step 3

The enrollment into the MERLIN study can occur at any time after having completed SABES Step 3, including immediately after the final visit of SABES.

10.3.1.2 New participants with acute HIV diagnosis

The new participants entering with acute HIV diagnosis will be enrolled as soon as possible after HIV diagnosis.

10.3.2. Follow-up Assessments

10.3.2.1 Participants from SABES step 3

The MERLIN study visits should occur at 24-week intervals with respect to SABES enrollment, rather than with respect to MERLIN enrollment in order to preserve time-points with respect to date of SABES enrollment and ART randomization

If the participant received MOH-based ART after completing SABES, the study visit schedule should resume a 24-weekly schedule with respect to timing from SABES enrollment, at the next possible visit (e.g. 72, 96, 120, etc., weeks).

10.3.2.2. New participants with acute HIV diagnosis

Participants who enroll into MERLIN immediately after acute HIV diagnosis will initiate ART treatment within 72 hours after the enrollment visit and will have follow-up visits following Peruvian clinical care guidelines.

10.3.3 Last study visit

The planned last study visit is not a particular numerical week, but rather will occur on or before December 31, 2022. All participants may remain involved in MERLIN for approximately 5 years, regardless of interval between the two studies.

10.3.4 Definitions of evaluations and special indications
10.3.4.1 Medical History

A medical history must be present in source documents. The medical history should be comprehensive and include HIV-1 related and non-related diagnoses. The medical history from SABES Step 3 should be reviewed and updated if appropriate. HIV uninfected and chronic HIV infection participants should have a medical history collected.

10.3.4.2 Medication History

A medication history must be present in source documents, including any history of antiretroviral medication, immune-based therapy, or HIV-1-related vaccines, including blinded study medications.

All prescription medications taken within 30 days prior to study entry, including actual or estimated start and stop dates, should be included in both the source documents and on the case report forms (CRFs).

All nonprescription medications taken within 30 days prior to study entry, including actual or estimated start and stop dates, should also be included in source documents.

10.3.4.3 Concomitant Medications

All of the concomitant medications taken since the last report should be registered in the source documents and the CRF and should include the start and end dates.

10.3.4.4 Modifications of Study Treatment Regimen

All of the pharmacologic modifications including the initial doses begun by the subject and/or interruptions proscribed by the protocol, modifications, and permanent suspension of the treatment should be recorded at ever visit in the source documents and the CRF

10.3.4.5 Clinical Assessments

1. Complete physical exam
   A complete physical exam is to be performed at study entry and study completion.

2. Targeted physical exam
   A targeted physical examination is required as clinically indicated.

3. Height to be collected at study entry.

4. Weight to be collected at all study visits.

5. Vital signs
   Oral temperature, pulse, and blood pressure to be collected at all visits.

6. Waist circumference should be measured at the enrollment (or the following visit) when the sample for lipid panel and glucose (fasting) is taken, as well as during week 144 and 216.

7. Signs and symptoms
   All signs, symptoms, and toxicities at entry must be documented in the subject's record and on the CRFs. Unless otherwise specified, after entry, only grade ≥ 3 and all signs and symptoms that led to a change in treatment, regardless of grade, must be recorded on the CRFs. The "Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December, 2004; Clarification August 2009" must be used and is available at http://rsc.tech-res.com/safetyandpharmacovigilance.

8. Diagnoses
   All confirmed and probable diagnoses made since the last visit must be recorded in the source documents and CRFs. The following diagnoses will be recorded in source

documentation and CRFs: U.S. Center for Disease Control and Prevention Category B and C diagnoses, major organ disease including but not limited to diabetes, cardiovascular disease and stroke, hepatitis, avascular necrosis, fat redistribution including central fat accumulation and peripheral fat wasting (as noted by subject and site staff and/or primary care provider). Please refer to AIDS Clinical Trials Group Appendix 60 for specific diagnoses and definitions which can be found at http://www.hptn.org/web%20documents/HPTN052/Appendix60V1.123Feb2007.pdf.

9. Neuropsychiatric Testing
   As part of the assessment of neuroinflammation in acute/recent HIV, each participant will undergo neuropsychiatric testing during each study visit. The visit will follow Study specific procedure (SSP) for neuropsychiatric testing.

10.3.4.6 Questionnaires

Questionnaires will be self-administered by participants at each visit (every 24 weeks). The questionnaire will take approximately 60 minutes in each visit.

This questionnaire will contain several modules:

1. Drug and alcohol use [drug use history, DAST 10 (drug abuse screening test) , drinking expectancies];
2. Mental health and well-being [RAND 12 (health well-beign) , Social support scale, Stigma scale, Stigma and diagnoses disclosure, Partner notification, Intimate partner violence, 10 item CES-D (depression scale), Mental health items on anxiety, Stress & Coping];
3. Medical care (hospitalization and ER use, Barriers to care, Access to care, Self-efficacy for ART use).

10.3.4.7 Laboratory Assessments

The “Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December, 2004; Clarification August 2009” must be used and is available at http://rsc.tech-res.com/safetyandpharmacovigilance.

All baseline laboratory values must be documented in the subject’s record and recorded on the CRFs. Subsequently, all laboratory values must be documented in the subject’s record, but unless otherwise specified, only laboratory values Grade ≥ **3** must be recorded on the CRFs. Any laboratory toxicity that led to a change in treatment, regardless of grade, must be recorded on the CRFs. Laboratory evaluations that do not have a grading scale in the “Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December, 2004; Clarification August 2009” must be collected on the CRFs if the values are outside the upper or lower limit of normal

1. Hematology
   Hemoglobin, white blood cell count, absolute neutrophil count, platelets;

2. Blood chemistries
   Serum amylase, lipase, bicarbonate, phosphorus, creatinine, AST (SGOT), ALT (SGPT),

alkaline phosphatase.

3. Lipid panel and glucose
   Subjects should be fasting for at least 8 hours for blood sample collection for lipid panel and glucose measurements. These tests will be performed in the enrollment visit and follow-up visits week 144 and 216. If the participant is not fasting, the tests must be deferred and will be taken during the next programmed visit.

   Triglycerides, total cholesterol, HDL, LDL and glucose.

4. Urinalysis
   Dipstick, and microscopic exam if dipstick is positive;
   Additionally urine samples will be stored for future drug analyses and STD tests.

5. Sexually transmitted infection testing
   Syphilis screening testing will be conducted by RPR and confirmed by MHA-TP if reactive. Syphilis diagnosis will follow Ministry of Health of Peru guidelines. A positive leukocyte esterase test by a urine dipstick will trigger a microscopic examination.

   Samples will be stored for further confirmation with a nucleic acid amplification test for *N. gonorrhoeae* and *C. trachomatis* if a diagnosis of urethritis is made. The results of these tests will be for research purposes only.

   Participants with a positive leukocyte esterase test and/or with symptoms will receive treatment following the local standard of treatment.

10.3.4.8 Immunologic Studies

1. CD4+/CD8+ T-cell subsets
   A CD4+ T-lymphocyte percent of total lymphocytes and CD8+ T-lymphocyte percent of total lymphocytes should be performed in addition to the absolute CD4+ and CD8+ T cell counts at screening and for all visits at which CD4+/CD8+ T cell counts are required. Because of the diurnal variation in CD4+ and CD8+ cell counts, determinations for individual subjects should be obtained consistently in either the morning or the afternoon throughout the study, if possible.

   NOTE: Each time a CD4+/CD8+ measurement is obtained, the local laboratory must perform a WBC and differential from a sample obtained at the same time.

10.3.4.9 Virologic Studies

1. Plasma HIV-1 RNA
   All HIV-1 RNA testing will be performed using the Abbott *m*2000 RealTi*m*e System (Abbott Molecular) at the IMPACTA PERU Clinical Trials Unit Central Laboratory, which is certified by the U.S. NIH/NIAID/DAIDS Virology Quality Assurance (VQA) Program. The baseline value will be the HIV-1 RNA level at the entry evaluation.

The results from the analysis of Plasma HIV-1 RNA at each 24-weekly visit will be available at the next follow-up visit to guide the medical management decision-making for each participant, according to the HIV management standards. Samples for HIV-RNA taken more frequently (from *SABES* timepoints) will not be used clinically and will only be used for research purposes and therefore will not be processed in real time.

2. HIV-1 genotype in stored samples will be performed by the University of Washington Virology Specialty Laboratory/Seattle Children's Hospital at the end of the study or when required per clinical judgment at time of suboptimal virologic response.

10.3.4.10 Leukapheresis

A subset of MERLIN participants (N = 40) will undergo leukapheresis. There is a separate consent form and only those who are newly enrolled and volunteer for this procedure will have it performed. Qualitative and quantitative assessment of the HIV reservoir (including its localization in different $CD4^+$ T cell subsets) will require large numbers of cells, which is important as both size and distribution of the HIV reservoir will be key factors in strategies to induce a functional cure. Thus, we will use leukapheresis to collect large samples at critical time points (*SABES* baseline and 24 weeks on all participants, then 72, 120, and 216 weeks for deferred arm, and 96, 144, and 196 weeks for immediate Arm).

10.3.4.11 Stored Plasma, PBMC, Whole Blood in RNA later

At all time points, whole blood, plasma, and PBMC will be stored for later analysis, many of which are described in the analysis plan, or else are for future studies as per specimen repository consents.

10.3.4.12 Stool Samples

Stool will be collected (25g per visit) at the visits indicated in the procedures table, for the evaluation of the microbiome throughout phases of VIH infection (acute-recent- and treated HIV infection) In one of the collaborative studies associated to this study, stool samples were collected. If these participants are enrolled in MERLIN they will be asked to provide stool samples in all the visits; however, for those participants who were not enrolled in the collaborative study and for who stool sample was not collected previously, only one sample will be requested during week 168 for the participants in the immediate SABES 3 arm, and during week 192 for those enrolled in the deferred SABES 3 arm.

10.3.4.13 Rectal Biopsies and Rectal Secretions

Rectal biopsies will be performed in a subset of 60 participants who additionally consent, using a separate informed consent procedure, to up to 4-3 rectal biopsies. Rectal secretions will be collected via a swab or sponge at the time of biopsy. In Immediate arm participants, the biopsies will be performed at 96, 144 and 192; ; in the Deferred arm, biopsies will occur at 120, 168 and 216 weeks. Biopsy procedure will be performed following the SSP.

10.3.4.14 Lumbar Puncture (LP)

A subset of up to 40 participants, ideally evenly dispersed between Immediate and Deferred Arms will undergo lumbar puncture to obtain cerebrospinal fluid (CSF). Those approached for CSF evaluation will sign a separate informed consent for this procedure and no MERLIN participants will undergo LP without signing a consent. LP will be performed in the specified visits as indicated in the table of procedures. Refer to the SSP for LP procedures.

11.WARNINGS AND PRECAUTIONS

11.1 LACTIC ACIDOSIS/SEVERE HEPATOMEGALY WITH STEATOSIS

Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogs alone or in combination with other antiretrovirals. A majority of these cases have been in women. Obesity and prolonged nucleoside exposure may be risk factors. Particular caution should be exercised when administering nucleoside analogs to any patient with known risk factors for liver disease; however, cases have also been reported in patients with no known risk factors.

Treatment with co-formulated EVG/COBI/FTC/TAF should be suspended in any patient who develops clinical or laboratory findings suggestive of lactic acidosis or pronounced hepatotoxicity (which may include hepatomegaly and steatosis even in the absence of marked transaminase elevations).

11.2 PATIENTS WITH HIV AND HBV COINFECTION

It is recommended that all patients with HIV be tested for the presence of hepatitis B before initiating antiretroviral therapy. Co-formulated EVG/COBI/FTC/TDF or EVG/COBI/FTC/TAF is not indicated for the treatment of chronic HBV infection and the safety and efficacy of these co-formulated regimenshave not been established in patients co-infected with hepatitis B virus and HIV. Severe acute exacerbations of hepatitis B have been reported in patients after the discontinuation of emtricitabine and tenofovir DF. Hepatic function should be closely monitored with both clinical and laboratory follow-up for at least several months in patients who discontinue co-formulated EVG/COBI/FTC/TDF or EVG/COBI/FTC/TAF and are co-infected with HIV and HBV.

HIV-1/hepatitis B co-infected individuals will not be enrolled in this study.

11.3 NEW ONSET OR WORSENING RENAL IMPAIRMENT

Renal impairment, including cases of acute renal failure and Fanconi syndrome (renal tubular injury with severe hypophosphatemia), has been reported with the use of tenofovir DF, and its co-formulations. Estimated creatinine clearance, urine glucose and urine protein should be documented in all patients prior to initiating therapy. Initiation of co-formulated EVG/COBI/FTC/TDFin patients with estimated creatinine clearance below 70 mL per minute is not recommended. Initiation of EVG/COBI/FTC/TAF in patients with estimate creatinine clearance <30 ml/min is not recommended.

Co-formulated EVG/COBI/FTC/TDF and co-formulated EVG/COBI/FTC/TAF should be avoided with concurrent or recent use of a nephrotoxic agent (e.g., high-dose or multiple non-steroidal anti-inflammatory drugs (NSAIDs)). Cases of acute renal failure after initiation of high dose or multiple NSAIDs have been reported in HIV-infected patients with risk factors for renal dysfunction who appeared stable on tenofovir DF. Some patients required hospitalization and renal replacement therapy. Alternatives to NSAIDs should be considered, if needed, in patients at risk for renal dysfunction. Persistent or worsening bone pain, pain in extremities, fractures and/or muscular pain or weakness may be manifestations of proximal renal tubulopathy and should prompt an evaluation of renal function in at-risk patients.

Routine monitoring of estimated creatinine clearance, urine glucose, and urine protein should be performed during co-formulated EVG/COBI/FTC/TDF or EVG/COBI/FTC/TAF therapy in all patients. Additionally, serum phosphorus should be measured in patients at risk for renal impairment.

Although COBI may cause modest increases in serum creatinine and modest declines in estimated creatinine clearance without affecting renal glomerular function, patients who experience a confirmed increase in serum creatinine of greater than 0.4 mg per dL from baseline should be closely monitored for renal safety.
The FTC, TAF, and TDF are primarily excreted by the kidney. Co-formulated EVG/COBI/FTC/TDF should be discontinued if estimated creatinine clearance declines below 50 mL per minute as dose interval adjustment required for emtricitabine and tenofovir DF cannot be achieved with the fixed-dose combination tablet. Co-formulated EVG/COBI/FTC/TAF should be discontinued if the creatinine clearance falls below 30 mL/min.

11.4 LIVER ENZYMES

In patients with known or suspected history of hepatitis B or C infection and in patients treated with other medications associated with liver toxicity, monitoring of liver enzymes is recommended. In patients with persistent elevations of serum transaminases to greater than five times the upper limit of the normal range, the benefit of continued therapy with co-formulated study treatment needs to be weighed against the unknown risks of significant liver toxicity.

Because of the extensive cytochrome P450 mediated metabolism of efavirenz and limited clinical experience in patients with hepatic impairment, caution should be exercised in administering co-formulated study treatments to these patients.

11.5 FAT REDISTRIBUTION

Redistribution/accumulation of body fat including central obesity, dorsocervical fat enlargement (buffalo hump), peripheral wasting, facial wasting, breast enlargement, and "cushingoid appearance" have been observed in patients receiving antiretroviral therapy. The mechanism and long-term consequences of these events are currently unknown. A causal relationship has not been established.

11.6 IMMUNE RECONSTITUTION SYNDROME

Immune reconstitution syndrome has been reported in patients treated with combination antiretroviral therapy., including the co-formulated study treatments. During the initial phase of

combination antiretroviral treatment, patients whose immune system responds may develop an inflammatory response to indolent or residual opportunistic infections (such as *Mycobacterium avium* infection, cytomegalovirus, *Pneumocystis jiroveci* pneumonia (PCP), or tuberculosis), which may necessitate further evaluation and treatment.

Autoimmune disorders (such as Graves' disease, polymyositis, and Guillain-Barré syndrome) have also been reported to occur in the setting of immune reconstitution, however, the time to onset is more variable, and can occur many months after initiation of treatment.

11.7 CO-ADMINISTRATION WITH RELATED DRUGS

Co-formulated EVG/COBI/FTC/TAF is indicated for use as complete regimens for the treatment of HIV-1 infection and co-administration with other antiretroviral products is not recommended.
Co-formulated EVG/COBI/FTC/TAF is not recommended for coadministration with the following:

- Product containing emtricitabine or tenofovir DF (ATRIPLA, COMPLERA, EMTRIVA, TRUVADA, VIREAD)
- Products containing lamivudine (COMBIVIR, EPIVIR, EPIVIR-HBV, EPZICOM, TRIZIVIR) or adefovir dipivoxil (HEPSERA)
- ritonavir (NORVIR, KALETRA)

12. MANAGEMENT OF TOXICITIES

12.1 CLINICAL TOXICITIES

Clinical AEs will be evaluated by the study physicians. Grade 3 and 4 clinical AEs will be referred to On-Site Principal Investigator at the time of the visit. A back-up coverage schedule will be developed and posted to assure that a study physician is available to the On-Site Principal Investigator responsible for medical decision-making, by phone at all times.

12.1.1 Nausea and Vomiting

12.1.1.1 Grade 1 or 2

Study treatment should continue without interruption. Subjects with Grade 1 and 2 nausea or vomiting may be treated symptomatically with permitted oral antiemetic therapies or antiemetic suppositories provided there is no suspicion of lactic acidosis or pancreatitis.

12.1.1.2 Grade ≥ 3

Subjects with Grade ≥ 3 nausea and vomiting should interrupt all study treatment until the toxicity grade returns to Grade 2 or to baseline. However, substitutions to the treatment regimen, as opposed to interruption of the entire regimen, may be made at the investigator's discretion upon consultation with the Study Chair. If the nausea does not improve with the substitution, then all study treatment should be interrupted. Once the toxicity returns to Grade 2 or baseline, study medications may be resumed. If Grade ≥ 3 nausea and vomiting recurs upon the resumption of study treatment, treatment should again be interrupted and alternative ART should be considered and discussed with the Study Chair. If Grade ≥ 3 nausea or vomiting persists for more than 4 weeks, then study treatment should be permanently discontinued.

12.1.2 Diarrhea

12.1.2.1 Grade 1 or 2

All subjects with study drug-related diarrhea should initially be treated symptomatically with antidiarrheal medications such as loperamide, diphenoxylate, and/or an aluminum hydroxide antacid. Study drug administration may continue at the discretion of the investigator.

12.1.2.2 Grade ≥ 3 Diarrhea

Grade ≥ 3 diarrhea that is unresponsive to antimotility agents should prompt interruption of all study treatment until resolution of diarrhea to Grade ≤ 2 or baseline. However, substitutions to the treatment regimen, as opposed to interruption of the entire regimen, may be made at the investigator's discretion upon consultation with the Study Chair. If the diarrhea does not improve with the substitution, then all study treatment should be interrupted. Once the toxicity returns to Grade 2 or baseline, study medications may be resumed. If Grade ≥ 3 diarrhea recurs upon the resumption of study treatment, all study treatment should be interrupted and alternative ART should be considered and discussed with the Protocol Chair. If Grade ≥ 3 diarrhea persists for more than 4 weeks, then study treatment should be permanently discontinued.

12.1.3 Symptomatic Hyperlactatemia

Occurrence of lactic acidosis is expected to be very rare with the medication regimen used in this protocol. However, if an investigator suspects – due to symptoms or lab abnormality – lactic acidosis or symptomatic hyperlactatemia, a lactate level should be drawn.

Symptomatic hyperlactatemia will be defined as new, otherwise unexplained and persistent occurrence for >2 weeks of one or more of the following in addition to an elevated lactate level:

- nausea and vomiting
- abdominal pain or gastric discomfort
- abdominal distention
- increased LFTs
- unexplained fatigue
- dyspnea
- motor weakness

If the lactate value is > 2 x ULN, obtain a confirmatory lactate value as soon as possible, preferably within 1 week. Study treatment should be discontinued immediately if the confirmatory value remains > 2 x ULN or if the site is unable to obtain a confirmatory value within 1 week. Determine lactate levels every 4 weeks until the lactate value returns to normal, at which time a new antiretroviral regimen may be constructed in consultation with the protocol team.

Management of symptomatic lactate value < 2 x ULN will proceed at the discretion of the local investigator. However, any modification of a subject's antiretroviral regimen should be made in consultation with the protocol team.

12.2 LABORATORY TOXICITIES

Laboratory results will be flagged by the laboratory as abnormal or critical using pre-determined criteria at the laboratory. Abnormal values will be confirmed by repeat testing of the same specimen. All critical values will be reported to On-Site Principal Investigator by cell phone or e-

mail. All results will be documented in laboratory records. All laboratory test results will be reported.

Participants who are not present at the study site at the time a critical value is identified will be contacted by telephone or in person and asked to come back to the study site for further evaluation, referral, and counseling.

Grade 3 and 4 laboratory abnormalities should be confirmed by repeat testing preferably within 3 calendar days of receipt of results and before study drug discontinuation, unless such a delay is not consistent with good medical practice. All laboratory abnormalities that would lead to a change in treatment should be confirmed as soon as possible.

12.2.1 Increases in Values for Liver Function Tests

12.2.1.1 Asymptomatic
For asymptomatic Grade 3 elevations of total bilirubin, AST, ALT, or alkaline phosphatase, study treatment may be continued at the discretion of the site investigator in consultation with the Study Chair. Alternatively, the investigator may make substitutions to the antiretroviral regimen upon consultation with the Study Chair.

If asymptomatic abnormalities in liver enzymes of Grade 3 persist for more than 4 weeks, the site investigator should consider temporary interruption of study treatment in consultation with the Protocol Chair.

12.2.1.2 Symptomatic or Grade 4
For symptomatic Grade 3 elevations of total bilirubin, AST, ALT, or alkaline phosphatase or for Grade > 3 elevations in AST, ALT, or alkaline phosphatase, all study treatment should be interrupted until the toxicity returns to Grade $\leq$ 2 or baseline, after which time all study treatment may be resumed.

If asymptomatic liver enzyme elevations Grade > 3 or symptomatic liver enzyme elevations persist for more than 4 weeks, despite the interruption of all study treatment, or if liver function abnormalities recur to Grade $\geq$ 3 at any time after the resumption of study treatment, then all study treatment should be interrupted or discontinued and the Study Chair should be contacted regarding the reintroduction of study treatment or the selection of alternate ART.

12.2.2 Lipase

12.2.2.1 Grade ≥ 2 Asymptomatic
If a Grade ≥ 2 elevation of lipase occurs, the subject should be evaluated for signs and symptoms of pancreatitis and additional diagnostic evaluation should be performed as clinically indicated. For asymptomatic Grade 2 or 3 elevations of lipase, study treatment may be continued at the discretion of the site investigator in consultation with the Protocol Chair. Alternatively, the investigator may choose to substitute another antiretroviral agent for any of the components of the original study regimen. If Grade $\geq$ 3 toxicity persists for more than 14 days despite interruption or substitution of study treatment, or recurs after reintroduction of study treatment, all study treatment should be permanently discontinued, and a new potent antiretroviral regimen should be

selected at the discretion of the local investigator when appropriate resolution of pancreatitis occurs.

Subjects with Grade 4 elevations in serum lipase without clinical symptoms of pancreatitis should have all study treatment interrupted until the toxicity returns to Grade < 1, then all study treatment or a substituted regimen may be restarted at full dose.

12.2.2.2 Symptomatic Pancreatitis (Regardless of Lipase Grade)
A diagnosis of pancreatitis should be considered if clinical symptoms such as nausea, vomiting, or abdominal pain are present. Subjects with these signs and symptoms should be evaluated for the presence of pancreatitis through the use of diagnostic testing, which should include serum amylase, lipase, and triglyceride measurements and may include pancreatic imaging via abdominal ultrasound and/or CT scanning as clinically warranted. If a diagnosis of pancreatitis is confirmed, all study treatment should be interrupted immediately.

12.2.3 Triglycerides/Cholesterol
Subjects with asymptomatic triglyceride elevations Grade > 3 may continue to receive study treatment at the discretion of the local investigator. In consultation with the Protocol Chair, the On-Site Principal Investigator may consider the addition of permitted lipid-lowering agents if necessary to treat hyperlipidemia. Dose interruptions or discontinuations of study treatment should be considered only for confirmed increased fasting triglycerides ≥ 1200 mg/dL.

Elevations in triglycerides ≥ 1200 mg/dL: For participants with this level of triglycerides, a fasting triglycerides level test will need to be performed and also a serum amylase and lipase. . For Grade ≥ 2 asymptomatic elevations in serum amylase and/or lipase (collected in response to elevated triglycerides) in the setting of Grade > 3 hypertriglyceridemia, subjects should be started on a fibrate, and amylase and lipase should be monitored every 2 weeks until amylase and lipase are Grade < 2. If symptoms of pancreatitis develop, study treatment should be interrupted until pancreatitis resolves, amylase, and lipase are Grade < 2 and hypertriglyceridemia is Grade < 3. Consideration should be given to continuing a fibrate if study treatment is reintroduced.

Subjects may continue on all study treatment despite Grade ≥ 3 triglyceride elevations if they remain asymptomatic with close clinical follow-up. In consultation with the Study Chair, the concomitant administration of gemfibrozil, clofibrate, or fenofibrate may be considered.

If Grade 4 triglyceride toxicity persists 4 weeks after the addition of medical intervention (addition of lipid-lowering agent), all study treatment should be interrupted. If Grade 4 toxicity persists for more than 14 days after all study treatment has been interrupted, consider permanent discontinuation of all study treatment and a new potent antiretroviral regimen should be selected at the discretion of the local investigator in consultation with the Study Chair.

12.2.4 Hyperglycemia
Appropriate dietary modifications and glucose-lowering agents should be initiated for subjects with hyperglycemia, at the discretion of the local investigator and in accordance with customary medical care.

12.2.5 Renal Insufficiency
Tenofovir DF substitution should occur for a confirmed creatinine clearance < 50 ml/min until an underlying etiology is determined. If no other etiology is determined or the renal insufficiency improves with holding tenofovir DF, tenofovir DF should be permanently discontinued.

Baseline serum creatinine will be the mean of the screening and entry values. If at any time serum creatinine is increased >1.5-fold above baseline, the serum creatinine should be repeated as soon as possible (preferably within one week). Participants with confirmed serum creatinine increases >1.5-fold above baseline should undergo an evaluation for potential causes of decreased renal function. Participants with confirmed increase in serum creatinine >1.5-fold above baseline should have serum creatinine monitored more frequently, at the discretion of the site investigator, until serum creatinine either stabilizes or decreases to ≤1.5-fold above baseline. Drug dosing adjustments should be done based on the calculated creatinine clearance.

12.2.6 Hypophosphatemia
For Grade 1 and Grade 2 hypophosphatemia, phosphorus should be repeated as soon as possible (within 2 weeks is optimal), and tenofovir DF may be continued without other signs of renal tubular acidosis at the discretion of the site investigator.

For asymptomatic Grade 3 decreases in phosphorus, subjects may either substitute another nucleoside reverse transcriptase inhibitor (NRTI) for tenofovir DF, or may remain on tenofovir DF with the following caveats. Subjects should be treated with oral phosphate supplements and phosphorus levels should be re-tested weekly until phosphorus returns to Grade 2 or better. If Grade 3 or worse phosphorus levels persist, tenofovir DF should be discontinued and another NRTI should be substituted. If Grade 4 hypophosphatemia occurs, tenofovir DF should be changed to an alternative NRTI in consultation with the Protocol Chair, and serum phosphorus levels should be followed weekly until resolution to Grade 2 or better.

12.3 ALL OTHER GRADE 3 OR 4 TOXICITY

Subjects who develop a Grade 3 adverse event or toxicity reasonably causally related to the study medications (with the exception of asymptomatic laboratory abnormalities) should have all their antiretroviral study medication held and the subject rechecked each week until the adverse event returns to Grade < 2. If the investigator has compelling evidence that the adverse event has not been caused by the study medication or has another acceptable management plan, dosing may continue after discussion with the Protocol Chair. Once the adverse event is Grade < 2, the medications may be reinstated at full dose. If the adverse event recurs after 4 weeks, the above management scheme may be repeated.

Subjects who develop a Grade 4 adverse event or toxicity (with the exception of asymptomatic laboratory abnormalities) should have all antiretroviral medication discontinued. The subject should be followed weekly until resolution of the adverse event and encouraged to complete the protocol study evaluations. Further management of antiretrovirals should proceed at the site investigator's discretion.

Subjects with Grade 3 or 4 asymptomatic laboratory abnormalities may continue therapy if the investigator has compelling evidence that the toxicity is not related to study drug, following discussion with the sponsor and the Protocol Chair.
The On-Site Principal Investigator or his designee has authority to stop study agent for any

participant due to clinical or laboratory AEs, and must stop study agent according to criteria defined in the safety monitoring section.

13. CRITERIA FOR DISCONTINUATION

13.1 PREMATURE STUDY DISCONTINUATION

- Request by the subject to withdraw;
- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the subject;
- Subject 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 Ministry of Health or National Institute of Health of Peru, any of the IRBs/ECs responsible for oversight of the study, study sponsor, or On-Site Principal Investigator.

13.2 PERMANENT TREATMENT DISCONTINUATION

- Drug-related toxicity (see section 9.0 Toxicity Management);
- Requirement for prohibited concomitant medications (see Section 5.3.3.);
- Clinical reasons believed life threatening by the Study Medical Director, even if not addressed in the toxicity management of the protocol;
- Suboptimal virologic response as defined in Section 4.4.;
- Subject repeatedly non adherent, to the extent that the physician believes it would not be in the subject's best interest to continue treatment;

The subjects who permanently discontinue treatment provided by the study according to the criteria described should continue a different ART regimen according to the standard of care currently in place. The study investigators will take necessary steps to ensure that the subjects continue on ART. Counseling will be provided with special emphasis on safety, and on efficacy of ART options to use in the future if the participant discontinues study ART, and does not continue with any other antiretroviral treatment.

13.3 ASSURING REPORTING OF SUSPENSION TO THE CLINICAL TRIAL

Any action resulting in a temporary or permanent suspension of the trial will be reported to the assigned and responsible NIDA Program Officer and Medical Officer.

14. STATISTICAL CONSIDERATIONS

14.1. PRIMARY OBJECTIVES

1) To determine the relative long-term benefits of immediate vs. early vs. delayed initiation of ART.

2) To determine the impact of high-level alcohol use on the relative long-term benefits of immediate vs. early vs. delayed ART initiation

14.2 SECONDARY OBJECTIVES

1) To estimate the impact of time of initiation of antiretroviral therapy on short- and long-term clinical and laboratory outcomes.

2) To determine whether NNRTI and INSTI-based ART regimens have differential effects on time to HIV VL suppression and size of the latent HIV reservoir

3) To determine whether NNRTI and INSTI-based ART have differential moderation of immune activation associated with HIV and alcohol abuse

4) To evaluate the correlation between gastrointestinal dysbiosis and systemic immune activation in MSM with HIV and alcohol use disorders

5) To evaluate the association with alcohol abuse and timing on ART initiation on neurocognitive function and neuro inflammation in MSM with acute and recent HIV

14.3 ENDPOINTS

14.3.1 Primary Endpoints

1) To determine the relative long-term benefits of immediate vs. early vs. delayed initiation of ART.

    1. Measure the impact of early treatment on cell-activation, and inflammation by comparing cellular markers of inflammation in study participants who initiated treatment at varying times after HIV acquisition.

    2. Measure the impact of early treatment on the replication-competent HIV reservoir (size of the reservoir and types of cells involved) in study participants who initiated treatment at varying times after HIV acquisition.

    3. Determine whether early ART initiation prevents long-term changes in gastrointestinal tract bacterial microbiota and correlate these changes with systemic markers of inflammation and disease progression such as the activity of the kynurenine pathway of tryptophan catabolism and plasma concentrations of the inflammatory cytokines.

    4. Document the impact of early ART initiation on ‘non-HIV-associated’ diseases

such as neurocognitive disorders. Assess the correlation of these clinical outcomes with laboratory markers of cell activation, inflammation and HIV reservoir size and characteristics.

5. Determine the impact of high-level alcohol use on the outcomes listed in 1-4, after controlling for time of ART initiation.

14.3.2 Secondary Endpoints

1. Examine the time course of seeding of the replication-competent HIV reservoir quantifying the size of the reservoir and identifying the types of cells involved at time-points close to HIV acquisition.

2. Characterize innate immune responses (cytokines and other soluble mediators) shortly after infection and determine the impact of these responses on the strength and character of the subsequent adaptive immune response to HIV, as well as the size and characteristics of the HIV reservoir (see above).

3. Probe changes in gastrointestinal tract bacterial microbiota shortly after HIV infection and determine the correlation of these changes with two established markers of disease progression: the activity of the kynurenine pathway of tryptophan catabolism and plasma concentrations of the inflammatory cytokines.

14.4 SAMPLE SIZE AND ACCRUAL

14.4.1 MERLIN COHORT OF ACUTE AND RECENT HIV INFECTION

Our goal is to enroll the total of participants who enrolled in the SABES step 3 study.

14.4.2 MERLIN SUB-STUDIES

40 participants will be separately consented and enrolled in sub-studies of qualitative integration of HIVreservoirs (leukapheresis) and neuro-inflammation; and 60 participants will also be separately consented for studies of GI microbiome (rectal biopsy/secretion). These participants may choose to participate in one, two, or all of the elective evaluations which require a separate consent and specific procedures as outlined in this protocol and corresponding SSPs.

14.5 MONITORING

All endpoints will be calculated and monitored on a quarterly basis throughout the period of enrollment and treatment.

14.6 ANALYSES

14.6.1 TESTING OF URINE, AND DRIED BLOOD SPOTS

Since high-level alcohol use is an exposure of interest, detailed questionnaire data will be collected and biologic specimens will be analyzed at least once a year to monitor use of alcohol and other substances (cocaine, marijuana).

14.6.2 MEASUREMENT OF THE HIV RESERVOIR

It is likely that early ART prevents the establishment of the long-lived latent reservoir, and this hypothesis is supported by several lines of evidence. However, to date almost all studies investigating this question have used PCR-based assays, which overestimate the size of the reservoir [12] because they do not distinguish between cells with defective, replication-incompetent cells vs. productively infected cells and the small pool of long-lived, latently infected cells. Conversely, the viral outgrowth assays measure only resting CD4+ T cells with replication competent HIV and likely underestimate the size of the reservoir [55]. Therefore, it is still unclear when and where the latent replication-competent reservoir is seeded. Our collaborator, Dr. Nicolas Chomont, has recently developed a novel assay (Tat/Rev Induced Limiting Dilution Assay, "TILDA") that can more precisely measure the frequency of productively and latently infected cells in a clinical sample as various times after infection. This assay measures the frequency of cells harboring inducible HIV without the above-mentioned limitations of standard assays (Figure 2). By combining ultrasensitive detection of multiply spliced (ms) RNAs upon T cell receptor stimulation together with a limiting dilution assay, this method allows Dr. Chomont's lab to measure a frequency of cells harboring transcriptionally silent - nonetheless inducible – viruses. To our knowledge, this viral induction assay is the only method that allows the measurement of the frequency of cells harboring inducible virus that can be performed with less than a million $CD4^+$ T cells.

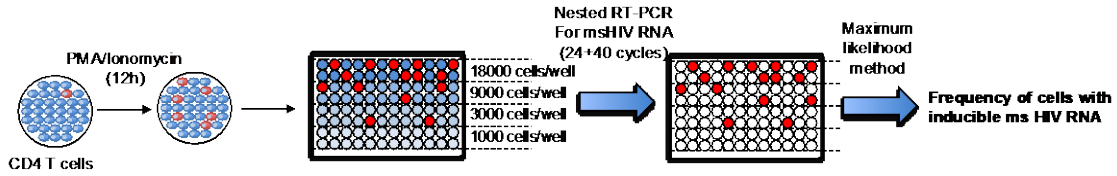

Figure 2: HIV RNA induction assay. Sorted $CD4^+$ T cells are stimulated for 12 hours with PMA/ionomycin. Serial dilutions of the stimulated cells are distributed in a 96-well plate and digested. Multi-spliced HIV RNA are detected in a 2-step real time PCR. The maximum likelihood method is used to calculate the frequency of cells with inducible HIV RNA based on the number of positive well.

14.6.3 INNATE IMMUNE RESPONSES AND INFLAMMATION

Innate immune cells are the major drivers of the inflammation that is required to mount protective immune responses and mount a rapid response after HIV acquisition [14]. The innate immune cells involved in this response are diverse in their phenotypes and functions. Cytokines secreted by these cells influence the maturation and development of effector cells that drive humoral and cellular immune responses to HIV. Molecular interactions of these cells with cells of the adaptive immune system also impact quantitative and qualitative features of primary and recall immune responses. It is likely that the balance among immune modulating factors induced shortly after HIV acquisition determines the strength and character of the adaptive immune response to infection as well as the size and characteristics of the HIV reservoir.

Using multiparameter flow cytometry we will: 1) enumerate T cells, B cells, monocytes, myeloid dendritic cells, plasmacytoid dendritic cells; and 2) perform in depth immunophenotyping of T cells, including markers of maturation (e.g., CD45RO/CD27), differentiation (e.g., into TH1, TH2, TH17, and/or Treg subsets), activation (e.g., Ki67, CD38, HLA-DR, and/or PD-1), and cytokine production (e.g., of IL-2, -4, -7, -16, TNF, and IFNγ). In addition, using plasma samples, we will measure markers of chronic inflammation (e.g., CRP, D-dimer, sCD14, and an array of chemokines and cytokines) using commercially available ELISA kits and multiplex assays, as well as activity of the immunomodulatory kynurenine pathway of tryptophan catabolism [kynurenine:tryptophan ratio] using LC-MS/MS as described previously. In addition, our collaborators, Drs. Rafick Sekaly and Mark Cameron, will use transcriptional profiling to measure changes in innate immune responses, cell activation markers and inflammatory mediators associated with HIV acquisition and in response to HIV infection and ART treatment. RNA-Seq transcriptomic profiling in his laboratory will provide an unbiased and sensitive tool to evaluate immune responses. This comprehensive approach will include the quantitative and qualitative assessment of the multiple pro- and anti-inflammatory pathways and their regulation; these parameters are not generated when performing conventional assays to measure cytokines.

14.6.4 DYSBIOSIS OF THE GUT MICROBIOTA AND CORRELATION WITH MARKERS OF HIV-ASSOCIATED IMMUNOPATHOLOGY.

Early HIV-associated damage to gastrointestinal mucosa and subsequent translocation of microbial products into the systemic circulation are thought to play a central role in the inflammatory changes associated with AIDS and non-AIDS related morbidities. Recent studies have shown that untreated HIV-infected subjects exhibit dysbiosis - a markedly different composition of the gut bacterial microbiota [19, 56]. This dysbiosis persists in some during ART, and the extent of dysbiosis correlates with activity of the kynurenine pathway of tryptophan catabolism and plasma concentrations of the inflammatory cytokine interleukin-6 (IL-6), two established markers of disease progression.

Stool specimens taken during untreated acute/recent infection, and after ART initiation will be processed and submitted to 16S rRNA-based phylogenetic characterization of microbiome composition in the laboratory of our collaborator, Dr. Susan Lynch. DNA will be extracted from stool and rectal biopsy/secretion samples using a protocol optimized for the isolation of bacterial DNA [57]. Exploratory data analyses will seek to identify operational taxonomic units (OTUs) that change in abundance as a result of acute HIV infection and to determine whether the abundance of such OTUs is linked to markers of immune activation and viral reservoir seeding. Furthermore, comparisons of the composition of distinct microbial community samples will be utilized to understand if initiation of early ART is associated with a gut microbiota that more resembles the

pre-infection state as compared to microbiota from subjects who initiate ART later after HIV acquisition. Multiple specimens from the same participant will be analyzed to assess how the timing of initiation of ART affects the long-term make-up of the GI microbiome. Urine will also be used to examine microbiome function through measurement of urinary metabolites.

14.6.5 DNA INTEGRATION SITES

Drs. Frenkel and Mullins will characterize and compare the HIV DNA integration sites in subjects with and without high-level alcohol use who started ART in Fiebig stages I-II, FIII-V and in the deferred ART arm. Among participants with sustained suppression of HIV RNA to undetectable levels, a total of 45 participants (~15 subjects from each group) will be studied upon enrollment (prior to the initiation of ART), and after 3.5 years of ART. PBMC from the subset of participants agreeing to leukapheresis will be preferentially selected for these studies.

14.6.6 NEUROLOGIC AND NEUROCOGNITIVE EFFECTS

We will use bi-annual neurocognitive testing to determine whether a) early ART initiation during acute or recent infection prevents cognitive decline and b) alcohol use during acute infection accelerates early cognitive decline caused by HIV. To determine the impact of early ART initiation and alcohol use on HIV infection in the CNS, neuronal injury and neuro-inflammation we will conduct analyses of cerebrospinal fluid (CSF) in a subset of 40 participants. These include measures of brain infection (CSF/blood viral load ratio), inflammation (soluble markers in blood/CSF) and injury (CSF markers of axonal degeneration) in subjects with and without alcohol use disorder at baseline and during follow-up.

15. DATA COLLECTION, MONITORING, AND ADVERSE EXPERIENCE REPORTING

15.1 DATA COLLECTION

Data collection in the MERLIN study will use the same questionnaires used for HIV+ participants in the SABES study. In addition, collection of medical data including medical record review, routine laboratory results, and results of routine and study-specific medical procedures (including laboratory tests, neurocognitive tests) will follow procedures established in the SABES study. Collection of biologic specimens will include blood collection for PBMC (peripheral blood mononuclear cells), plasma and serum, urine, GI biopsies and stool. Because drug and alcohol use are common among HIV-infected individuals and individuals at high risk of HIV infection, we will collect detailed information on drug and alcohol use as well as collecting dried blood spots, hair and urine for tests for drug and alcohol metabolites.

Stored blood samples will be used in assays measuring the HIV reservoir (size and cellular location), and biologic responses to HIV infection and ART treatment including measures of cell activation, markers of inflammation, innate immune cell signaling and induced immune responses. Stool samples will be collected for assessment of the gastrointestinal microbiome to support studies of HIV-induced dysbiosis and how the microbiome influences systemic inflammatory markers thought to play a prominent role in HIV immunopathogenesis. HIV viral load will be monitored in plasma. Specimens will be used for development of new assays for detections of early HIV infection; this will involve plasma at a minimum but may involve additional specimen types as well.

15.2 DEFINITION OF AN ADVERSE EVENT

An adverse event (AE) is defined as any untoward medical occurrence in a clinical research participant who has been administered an investigational product, and that does not necessarily have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the product. This definition will be applied to both study treatment groups, even though one group is assigned to receive an investigational product immediately and the other not, and will be applied to both groups beginning from the time of random assignment.

Study participants will be provided instructions for contacting the study site to report any untoward medical occurrences they may experience, except for possible life-threatening events, for which they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where the study clinician is based, and to request that the clinician be contacted upon their arrival. With appropriate permission of the participant, and whenever possible, records from all non-study medical providers related to untoward medical occurrences will be requested and required data elements will be recorded on study case report forms and/or in the participant's medical chart.

15.3 GOVERNANCE

The Protocol Chair is ultimately responsible for adherence to all aspects of the study protocol, including reporting of AEs. This responsibility is largely delegated to the On-Site Principal Investigator, who is responsible for medical decisions. He or his authorized study physicians (all of them trained in internal medicine and/or infectious diseases) will prepare all AE reports. In addition, there is real-time data checking by the data management unit at the study site, which will review data collection forms, including clinic and laboratory reports, from all participants. The review will serve to assure that evidence of AEs is identified and reported.

A Protocol Safety Review Committee will review AE reports and determine appropriate follow-up, including modifying the study protocol and/or procedures as necessary.

15.4 ASSESSMENT OF AE RELATIONSHIP TO STUDY AGENT

The on-site Principal Investigator or his designee will assess whether an AE is related or not related to the study agent, using the available information about co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate, co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide,and his/her clinical judgment.

The anticipated risks associated with this study are deemed overall to be low.

The assessment of risk does not, however, preclude the potential for anticipated and/or unanticipated AEs, serious or otherwise, since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods.

We will use a Data Safety Monitoring Plan (DSMP) to monitor the overall progress of our study. Some of the factors the investigators intend to monitor closely include: 1) Are the treatment groups comparable at baseline; 2) Are the accrual rates meeting initial projections and is the trial on its

scheduled timeline; 3) Are the data of sufficient quality; 4) Are the treatment groups different with respect to safety and toxicity data; 5) Are the treatment groups different with respect to efficacy data; 6) Is there an indication to discontinue the trial due to predefined criteria; and 7) Should the protocol be modified in response to any unanticipated outcome or change in temporal event?.

15.5 FOLLOW-UP OF ADVERSE EVENTS

All participants reporting an untoward medical occurrence will be followed clinically until the occurrence resolves (returns to baseline) or stabilizes. Laboratory AEs will be managed according to established protocols described in 9.0 Toxicity Management. Laboratory results that require action will be documented and a peer navigator will be contacted to schedule a repeat visit.

15.6 EXPEDITED ADVERSE EVENTS REPORTING TO NATIONAL INSTITUTE OF DRUG ABUSE

The expedited adverse event (EAE) reporting requirements and definitions for this study and the methods for expedited reporting of AEs to the NIDA will be based on “The Manual for Expedited Reporting of Adverse Events to DAIDS Version 2.0” dated in January 2010. This manual is available on http://rsc.tech-res.com/safetyandpharmacovigilance. Adverse events reported on an expedited basis must be documented on the 2010 NIDA Serious Adverse Event form and reported to the NIDA Program Official within 72 hours by fax (1-301-443-6814) and/or email. Investigators will submit a written report to the NIDA Program Official no more than three days after site aware. Written follow-up will be sent to the NIDA Program Official

This study uses the standard level of EAE reporting. In addition, the On-Site Principal Investigator will submit AE information as required by local regulatory or other relevant authorities.

15.6.1 Study Agents for Expedited Reporting to National Institute on Drug Abuse Program Official

The study drugs on which the relatedness assessment to be based are co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate or elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide and reported to the NIDA Program Official.

15.6.2 Grading Severity of Events

All AEs will be graded using the “Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December, 2004; Clarification August 2009” available at http://rsc.tech-res.com/safetyandpharmacovigilance.

15.6.3 Adverse Events Reporting Periods

Adverse events must be reported on an expedited basis at the Standard Level during the protocol-defined EAE reporting period, which is: The entire study duration for an individual subject (from study enrollment until study completion or discontinuation of the subject from study participation for any reason). After the end of the protocol-defined EAE reporting period stated above, the study site must report serious, unexpected, clinically suspected AE drug reactions if the study site staff becomes aware of the event on a passive basis, i.e., from publicly available information.

15.7 CONTACT FOR QUESTIONS ABOUT EXPEDITED ADVERSE EVENTS

For questions about EAE reporting, contact NIDA Program Official sending an e-mail.

15.8 SOCIAL ADVERSE EVENTS

This study will be designed to minimize risk and maximize benefit to both study participants and their local communities. However, volunteers could experience discrimination or other personal problems as a result of being in the study. We will provide advocacy for and assistance to participants regarding negative social impacts or social AEs associated with the study.

Social AEs may include loss of privacy, stigmatization, interference with gainful employment, and coercion. Information regarding social AEs will be solicited during quarterly study visits after enrollment in the study and will be recorded in log form on case report forms. In addition, social harms may be identified by other study staff, including peer navigator, patient advocates, nurses, physicians, pharmacists, and others. All social adverse events will be brought to the attention of the Study Medical Director and reported according to AE reporting guidelines. Participants who report social harms will be referred to speak with a social worker and/or a study counselor.

16. HUMAN SUBJECTS PROTECTION

16.1 INSTITUTIONAL REVIEW BOARDS AND ETHICS COMMITTEE REVIEW

This protocol and the informed consent forms and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study. Signed consent forms will be obtained from the subject. The consent forms will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent forms will be given to the subject, and this fact will be documented in the subject's record.

16.2 SUBJECT CONFIDENTIALITY

All laboratory specimens, evaluation forms, reports and other records that leave the site will be identified by coded number only to maintain subject 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 subject, except as necessary for monitoring by the Ministry of Health or National Institute of Health of Peru, any of the IRBs/ECs responsible for oversight of the study, or the NIDA.

16.3 STUDY DISCONTINUATION

The study may be discontinued at any time by the Ministry of Health or National Institute of Health of Peru, any of the IRBs/ECs responsible for oversight of the study, or NIDA as part of their duties to ensure that research subjects are protected.

17. PUBLICATIONS OF RESEARCH FINDINGS

Presentation and publication of the results of this study will follow the regulations of Fred Hutchinson Cancer Research Center and Asociacion Civil Impacta Salud y Educacion. Any presentation, abstract, or manuscript must be approved by the Protocol Chair prior to submission.

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Amendment Letter N° 01

Date : July 31 2018

To : Research Protocol Team

From : Protocol Chair and Co-Chair

Topic : Amendment letter N° 1 to MERLIN Protocol, Version 3.0, April 12 2018, entitled "Modulating the Impact of Critical Events in Early HIV Infection: Effect of ART Initiation and Alcohol use".

This amendment letter will be sent to the respective Ethics Committees. Each site will need to file a copy of this amendment letter together with the protocol of reference. The amendments contained in this letter must be implemented immediately after approval by the Peruvian National Institute of Health. These updates will be included in the next version of the protocol if applicable.

The changes proposed in this letter are aligned with the main primary and secondary objectives of the study protocol, which are not being modified.

- **Section 10.3.3 Last study visit.** This section has been amended to include termination of participants by consideration of the protocol team, it now reads:

  The last study visit will not be a specific numeric visit, but will occur before or by December 31 2022. All participants will be followed for approximately 5 years independently of the interval between the SABES and MERLIN studies, **with the exception of those participants who, by the protocol team decision, must be terminated (having a shorter follow up time).**

- **Section 13.1 Premature termination from the study.** A new category has been added, it now reads:

  - Protocol team request, analysis participant data/specimens will not be important of the study evaluation (they will not be relevant for the statistical analysis).

- **Section 13.4 Follow-up of participants without specimen collection different from the required for safety labs to evaluate treatment.** This section has been included to describe the follow-up of participants for whom the different specimen collections for storage will be no longer done, it now reads:

  Participants who by protocol team evaluation are in the following categories:

  - Without antiretroviral treatment for a period longer than 3 months; however having undetectable viral load values
  - Viral load levels >1000 copies/mL without treatment interruption (good adherence)
  - Do not have PBMC, stool, urine, CSF since HIV diagnosis

Amendment Letter N° 02

Date : March 01 2019

To : Research Protocol Team

From : Protocol Chair and Co-Chair

Topic : Amendment letter N° 2 to MERLIN Protocol, Version 3.0, April 12 2018, entitled "Modulating the Impact of Critical Events in Early HIV Infection: Effect of ART Initiation and Alcohol use".

This amendment letter will be sent to the respective Ethics Committees. Each site will need to file a copy of this amendment letter together with the protocol of reference. The amendments contained in this letter must be implemented immediately after approval by the Peruvian National Institute of Health. These updates will be included in the next version of the protocol if applicable.

The changes proposed in this letter are aligned with the main primary and secondary objectives of the study protocol, which are not being modified.

- **Section 10.3.4.10 Leukapheresis.** This section has been amended to increase the number of participants that could undergone the procedure, it now reads:

  All the study participants could be invited to undergo leukapheresis. Based on operational and budget considerations it was initially considered to include only 40 participants for this procedure. However, we have lost participants who initially had accepted to undergo this procedure, because they are no longer eligible to continue in the study or because they refuse to continue in the procedure for personal reasons.

  This modification will allow replacement of participants who could have been lost to follow-up in MERLIN, and include additional participants who are eligible for these procedures. This will allow a more robust analysis at the late time points of the study.

  Participants will be invited to participate in this procedure according to their antiretroviral treatment initiation condition and clinical evolution.

  There is a separate **informed** consent. The qualitative and quantitative evaluation of HIV seeding (including its location in different CD4+ T cells subsets) will require a big number of cells, and it is important to learn about the size and distribution of the HIV reservoir, this is a key factor to induce a functional cure. Hence, we will use leukapheresis to collect a big number of cells at specific times (weeks 72, 120, 168 and 216 for the deferred arm and weeks 96, 144, and 192 for the immediate arm).

- **Section 10.3.4.14 Lumbar Puncture.** This section has been amended to increase the number of participants that could undergone the procedure, it now reads:

  All the participants could be invited to undergo lumbar puncture. Based on operational and budget considerations it was initially considered to include only 40 participants for this procedure. However, we have lost participants who initially had accepted to undergo this

procedure, because they are no longer eligible to continue in the study or because they refuse to continue in the procedure for personal reasons.

This modification will allow replacement of participants who could have been lost to follow-up in MERLIN, and include additional participants who are eligible for these procedures. This will allow a more robust analysis at the late time points of the study.

Participants will be invited to participate in this procedure according to their antiretroviral treatment initiation condition and clinical evolution.

Those participants who accept the procedure will need to sign a specific informed consent; otherwise, no procedure could be performed. The evaluations will be done at specific visits indicated in the procedures table.

Amendment Letter N° 03

Date : December 1 2019

To : Protocol Research Team

From : Protocol Chair and Co-Chair

Topic :Amendment Letter N° 3 to Protocol MERLIN, Version 3.0, April 12 2018, entitled "Modulating the Impact of Critical Events in Early HIV Infection: Effect of ART Initiation and Alcohol use".

This amendment letter will be sent to the respective Ethics Committees. Each site will need to file a copy of this amendment letter together with the protocol of reference. The amendments contained in this letter must be implemented immediately after approval by the Peruvian National Institute of Health. These updates will be included in the next version of the protocol if applicable.

The changes proposed in this letter are aligned with the main primary and secondary objectives of the study protocol, which are not being modified.

- **Section 1.1.2. Asociación Civil Impacta Salud y Educación.** This section has been modified to update the local study coordination, it now reads (insertion in bold):

  Maria Mamani, CNM
  Study Coordinator
  IMPACTA PERU Clinical Trials Unit
  Asociación Civil Impacta Salud y Educación
  Av. Almirante Miguel Grau No. 1010. Lima 4, Peru.
  Phone: +51-1-206-7800 **Ext 209**
  e-mail address: **mmamani@impactaperu.org**

- **Section 10.1. VISIT PROGRAM.** This has been modified to update, based on considerations explained below, that in the annual visits (every 48 weeks) after week 192 for participants assigned to the immediate arm, and after week 216 for those assigned to initiate deferred treatment (both, after diagnosis of acute HIV infection):

  1. Analysis of CD4+/CD8+ T cell subpopulations will not be performed
  2. No PBMC collection will be performed
  3. The volumen of blood to be collected in such visits has been updated (97 mL instead of 250 mL)

  These changes bring as a consequence the modification of the informed consent: "Informed Consent to be enrolled in the MERLIN study".

- **Section 10.1. VISIT PROGRAM.** This section has been modified to update, based on considerations described below, that in the bi-annual visits after week 192 for participants assigned to the immediate arm, and after week 216 for those assigned to initiate deferred treatment (both, after diagnosis of acute HIV infection):

  1. Analysis of CD4+/CD8+ T cell subpopulations will not be performed

  These changes bring as a consequence the modification of the informed consent: "Informed Consent to be enrolled in the MERLIN study".

- **Section 10.3.4.8. Immunologic Studies,** item 1. CD4+/CD8+ T-cell subsets. It has been modified to describe the most current medical care standard offered to people living with HIV in our country, it now reads (insert in bold):

  A CD4+ T-lymphocyte percent of total lymphocytes and CD8+ T-lymphocyte percent of total lymphocytes should be performed in addition to the absolute CD4+ and CD8+ T cell counts at screening and for all visits at which CD4+/CD8+ T cell counts are required. Because of the diurnal variation in CD4+ and CD8+ cell counts, determinations for individual subjects should be obtained consistently in either the morning or the afternoon throughout the study, if possible.

  As described in the most current "Technical Standard of Health for Comprenhensive Care of Adults living with Human Deficiency Virus" of the Peruvian Ministry of Health dated 2018, the evaluation of CD4+/CD8+ subsets is not considered as part of the standard medical care offered to people living with HIV under stable antiretroviral treatment, because it is a priority to monitor levels of HIV-1 RNA in plasma for its impact determining alternative therapies in the absence of viral suppression. Based on technical, operational and budget considerations, during the annual and bi-annual visits after week 192 for participants assigned to the immediate antiretroviral treatment initiation arm and after week 216 for those assigned to initiate deferred antiretrovrial treament (both after being diagnosed with acute HIV infection), no evaluation of CD4+/CD8+ subsets will be performed, unless there is a justified medical indication to perform such evaluation.

  NOTE: Each time a CD4+/CD8+ measurement is obtained, the local laboratory must perform a WBC and differential from a sample obtained at the same time.

- **Section 10.3.4.11 Stored Plasma, PBMC and Whole Blood in RNA Later.** It has been modified to indicate that PBMC will not be collected in the respective visits, based on operational and budget considerations, it now reads (insertions in bold):

  At all time points, whole blood, plasma, and PBMC will be stored for later analysis, many of which are described in the analysis plan, or else are for future studies as per specimen repository consents.

  Based on technical, operational and budget considerations, during the annual and bi-annual visits after week 192 for participants assigned to the immediate antiretroviral treatment initiation arm and after week 216 for those assigned to initiate deferred antiretroviral treatment (both after being diagnosed with acute HIV infection), no PBMC collection will be collected, because it will not contribute with the evaluation of the main study objectives which is planned for years 2 and 4 after initiation of antiretroviral treatment.