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HIV Testing and Treatment to Prevent Onward HIV Transmission among MSM and Transgender Women in Lima, Peru

Project "¿SABES?"

Sponsored by:

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

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2 SUMMARY

Protocol Title: HIV Testing and Treatment to Prevent Onward HIV Transmission Among MSM and Transgender Women in Lima, Peru.

Short Protocol Title: Test and treat early HIV among MSM and Transgender Women in Lima, Peru

Study Name: "¿SABES?"

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

Planned Starting Date: July 2012

Planned Ending Date: July 2016

Study Rationale: This research will expand upon Seek-Test-Treat-Retain (STTR) strategies based on HIV testing and treatment of individuals with acute and recent HIV infection. The overall objective is to improve the efficacy of STTR strategies by specifically intervening to reduce the impact of viral load on HIV transmission among individuals with acute and recent HIV infection. This research will be conducted in Lima, Peru. We will investigate the population-level impact of drug and alcohol use on HIV transmission by examining the role of men who have sex with men (MSM) and transgender women who report substance use (mostly alcohol) in transmission clusters identified through partner tracing and phylogenetic analysis. This analysis will investigate the feasibility of incorporating tests for acute HIV infection into routine HIV testing and will model the contribution of men with acute and recent HIV infection to transmission among networked populations, such as MSM and transgender women.

The overall aim of this project is to model STTR strategies that specifically intervene to reduce HIV transmission through reductions in HIV-1 viral load and sexual risk behavior among individuals with early HIV infection, including both acute and recent infections.

Primary Objectives

1. To estimate the impact of immediate and deferred (24 weeks after study enrollment) antiretroviral therapy (ART) on the decay dynamics of HIV viral load in plasma, semen and rectal secretions from individuals with early HIV infection;

2. To investigate the impact of drug and alcohol use on HIV transmission by examining and modeling the role of MSM and transgender women with substance abuse disorders in transmission clusters identified through partner tracing and phylogenetic analysis.

Secondary Objectives

1. To determine HIV incidence and associated risk factors among high-risk HIV-1 uninfected MSM and transgender women who participate in a program of frequent routine HIV testing.

2. To attempt to identify transmission clusters in phylogenetic trees constructed using early viral specimens from study participants with acute or recent HIV infection.

3. To determine other characteristics (sexual risk behavior, partner characteristics, use of entertainment venues, and reason for HIV testing) in MSM and transgender women within clusters compared to those outside of clusters.

4. To assess the feasibility and acceptability of re-contacting HIV-negative individuals with evidence of acute infection (HIV-1 RNA positive) for further HIV testing and linkage to care.

5. To evaluate the frequency of HIV testing among recent sexual partners of participants diagnosed with early HIV-1 infection, comparing strategies such as partner tracing and notification, and general and targeted community outreach.

6. To evaluate retention in care, including adherence to ART and study procedures, among individuals with early HIV-1 infection receiving immediate and deferred ART.

Primary endpoints

1. To quantify HIV viral load in plasma, semen and rectal secretions in individuals with early HIV infection in immediate versus deferred treatment groups.

2. To identify transmission clusters through phylogenetic analysis among participants with early HIV infection receiving immediate and deferred ART; Construction of models to assess the potential population impact of a seek-test-treat-retain strategy.

Secondary endpoints

1. To identify risk factors associated with incident HIV-1 infection.

2. To identify sequence clusters using phylogenetic analysis among participants with early HIV infection.

3. To compare sexual risk behavior, partner characteristics, entertainment venue attendance, and method of referral in MSM and transgender women within clusters to those outside of clusters.

4. To determine the proportion of individuals with evidence of acute infection (HIV-1 RNA-positive) who are successfully contacted for confirmatory HIV testing and are then linked to care.

5. To determine the proportion of newly identified HIV-positive men willing to name at least one recent sexual partner, and the proportion of named partners subsequently contacted and tested.

6. To determine the proportion of men retained in care, including adherence to ART and study procedures, among individuals with early HIV-1 infection receiving immediate and deferred ART.

Design: This is three-step trial. In Step 1, we will screen MSM and transgender women, unaware of their HIV status and with evidence of high risk for acquiring HIV-1 infection, including risky sexual behavior, a partner of a newly-diagnosed person with acute or recent HIV

infection or with symptoms of acute retroviral syndrome. We anticipate testing a approximately 2400 MSM and transgender women until July 2015. In Step 2, high risk HIV-1 uninfected MSM with high risk sexual behavior will be tested at variable times (during which they will receive standard HIV prevention education) for incident HIV-1. Roughly, we anticipate re-testing approximately 800 MSM and transgender women per month until July 2015. In Step 3, individuals with acute and recent HIV-1 infection detected at Step 1 or 2; or individuals who have been diagnosed with HIV in any health clinic or laboratory authorized by the Ministry of Health, and that have had a documented negative result in the past three months, will be enrolled in a 48-week randomized, open label clinical trial of the effects on the decay dynamics of HIV viral load in plasma, semen and rectal secretions of immediate vs. deferred (by 24 weeks) ART, unless ART initiation criteria are met earlier. We expect approximately 200-250 MSM and transgender women (roughly 100-125 individuals with acute and 100-125 with recent HIV-1 infection).

Those participants, who have started their participation in the study before the approval of **Amendment Letter #1** in this study protocol, will continue their participation as originally programmed.

Once the **Amendment Letter #1** is approved for this study protocol, the participants who are eligible for Step 3 will be given treatment the following way: 1) those people diagnosed with **acute HIV infection** will be offered immediate ART (just at time of the enrollment visit or during the next 48 to 72 hours) for the duration of 48 weeks. 2) The people who are diagnosed with **recent HIV infection** will be randomized to start immediate ART or defer ART initiation until 24 weeks after HIV diagnosis, all of them followed for 48 weeks after which they will continue ART from other sources.

Intervention: This is a study of timing of antiretroviral therapy(ART) initiation. This study will not compare one ART medication to another .The ART medications that will be used can be taken as one pill a day so that number of pills taken each day is not a burden for people in the study. Co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (STRIBILD®) or emtricitabine/tenofovir disoproxil fumarate/efavirenz (Atripla™). When STRIBILD and Atripla™ are not available, co-formulated Truvada™ (emtricitabine 200 mg / tenofovir disoproxil fumarate 300 mg) plus efavirenz 300 mg may be used. Elvitegravir is an HIV integrase strand transfer inhibitor (HIV-1 INSTI), cobicistat, a CYP3A inhibitor, and emtricitabine and tenofovir DF are both HIV nucleoside analog reverse transcriptase inhibitors (NRTI)and efavirenz is a non-reverse transcriptase inhibitor. All these medications have been licensed for the treatment of HIV-1 infection in humans by the U.S. Food and Drug Administration (FDA).

Duration: In Step 1, volunteers will have a cross-sectional evaluation. In Step 2, study participants will have variable times of participation in re-testing until July 2015. In Step 3, individuals with acute and recent HIV-1 infection detected at Step 1 or 2 will be followed for 48 weeks.

Inclusion/Exclusion Criteria:
In Step 1, potential participants must be MSM and transgender women , have sex with men, HIV status, at high-risk for HIV acquisition. Each potential participant must have the ability and willingness to provide informed consent for study participation, including being tested for HIV-1. If HIV infection is not diagnosed, he must be willing to be enrolled in Step 2, and if diagnosed with acute or recent HIV infection be willing to consider participation in Step 3. In particular, if they are HIV seronegative on antibodies analysis, but with evidence of acute infection (HIV-1

RNA-positive confirmed by HIV antigen analysis), they should be willing to be re-contacted for further HIV testing and linkage to care.

In Step 2, volunteers who were found to have no HIV infection from Step 1 will be re-tested at variable times for incident HIV-1. Roughly, we anticipate testing 800 MSM and transgender women per month and follow-up until July 2015.

In Step 3, men with acute or recent HIV-1 infection detected at Step 1 or Step 2; or individuals who have been diagnosed with HIV in any health clinic or laboratory authorized by the Ministry of Health, and that have had a documented negative result in the past three months with no prior ART exposure, with normal hematological, renal and liver function, will be enrolled in a randomized clinical trial for immediate vs. deferred ART, unless ART initiation criteria are met earlier.

Treatment Regimen: In Step 1 and Step 2, no study treatment is offered. All participants will receive standard risk reduction counseling, condoms, and management of STIs. In Step 3, subjects will receive treatment as described above.

Two ART regimens are available:

- STRIBILD, co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate
- ATRIPLA,emtricitabine/tenofovir,DF/efavirenz

Co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate will be the preferred regimen for newly enrolled participants because of the early benefit of integrase inhibitors on the decline of plasma viraemia and co-formulated emtricitabine/tenofovir DF/efavirenz will be an alternative regimen. Participants who have already initiated therapy with emtricitabine/tenofovir DF/efavirenz will continue on this regimen unless they develop clinical indications warranting change in medication.

Evaluations: In Step 1, volunteers will have a cross-sectional evaluation. In Step 2, all participants will be tested quarterly. In Step 3, all participants will be evaluated at regular intervals for primary and secondary outcomes.

3 BACKGROUND

3.1 INTRODUCTION [1]

Despite two decades of HIV preventive initiatives the global prevalence of HIV continues to rise. At the end of 2010, an estimated 34 million people were living with HIV worldwide, up 17% from 2001. This reflects the continued large number of new HIV infections and a significant expansion of access to antiretroviral therapy (ART), which has helped reduce AIDS-related deaths, especially in more recent years [2].

Currently, HIV-prevention strategies are only partly effective, condoms remain severely underused, no cure is available, and the discovery of an effective preventive vaccine remains elusive. Randomized controlled trials (RCTs) have provided evidence that male circumcision reduces the risk of heterosexual men becoming infected with HIV [3-5]. A glimmer of hope comes from the CAPRISA 004 trial which recently reported that a 1% vaginal gel formulation of tenofovir reduces HIV acquisition by an estimated 39% overall, and by 54% in women with high gel adherence [6], and as well as promising results from recent pre-exposure prophylaxis trials using antiretrovirals to prevent HIV among populations at risk [7-10]. Despite these important findings, the international community needs to think about additional effective prevention strategies in order to try to suppress the spread of HIV.

One potential approach to prevention is to build on the success of antiretroviral therapy, which has effectively suppressed HIV viral load of those on treatment [11, 12] and which appears to lead to a substantial reduction in HIV transmission [13-17]. **This proposed 'test-and-treat' approach involves very high levels of population coverage of frequent HIV testing and immediate initiation of ART upon diagnosis.** While the potential strategy has received attention for its possible adoption in developing countries, there is also interest in its application in developed countries.

3.2 RATIONALE

Transmission of HIV from an infected person to an uninfected one occurs through exposure to the infected person's bodily fluids, mainly semen, vaginal secretions, breast milk or blood [18-20]. HIV transmission risk in unprotected sexual intercourse depends on a number of factors, including the type of sexual practice [21], the stage of HIV infection of the infected partner [22-25], the presence of sexually transmitted infections [22], the susceptibility of the uninfected partner [26] and HIV RNA viral load in the infected partner [13, 27, 28]. This last factor in particular has been shown to be the main predictor of the risk of sexual transmission of HIV-1 [27].

HIV testing is fundamental to HIV prevention, treatment, and care. Studies have showed that those who are found positive modify their behavior in order to reduce the risk of HIV transmission [29-31]. This is likely to be particularly the case for sex with long-term partners. Widespread use of ART has led to a dramatic decrease in morbidity and mortality among HIV-infected patients both in low-income and developed countries [32-35]. Antiretroviral therapy reduces levels of HIV-1 RNA in infected persons, and reduced levels have been measured in plasma [15, 36], female genital tract secretions [37], semen [12] and rectal secretions [38]. Due to the strong association between plasma viral load and risk of transmission, there is the potential for ART to prevent sexual transmission of HIV by reducing the infectiousness of HIV-infected patients [27], and there is increasing evidence to support this [13-17]. There is much

support for the principle that extension of ART treatment could play a role in curbing the advance of the HIV/AIDS pandemic, but many [39-43] have doubts over such a strategy, citing concerns such as whether it could be practically implemented at a level sufficient to produce a significant impact on incidence, whether it should be implemented due to inherent ethical difficulties, and whether it would be cost-effective.

Evidence of effectiveness of ART in preventing HIV transmission comes from different sources: Use of ART to prevent mother-to-child transmission (MTCT) [44-46], studies on sero-discordant heterosexual couples [13-15, 17, 25, 27] and ecological studies [47-49]. Randomized clinical trials have demonstrated that reducing maternal plasma HIV-1-RNA concentrations with ART drastically reduces MTCT [44-46]. Several studies of HIV sero-discordant heterosexual couples [14, 15, 17, 25, 27] have reported that transmission is rare in patients on ART, particularly in those with low HIV-1 RNA concentrations. A recent meta-analysis [13] on the risk of HIV transmission through unprotected sexual acts according to HIV-1-RNA concentrations and treatment with ART showed that among heterosexual HIV sero-discordant couples no transmissions were observed in patients treated with ART and with HIV-1RNA levels below 400 copies/ml, and that data were compatible with one transmission per 79 person-years. A word of caution comes from a Chinese study of sero-discordant couples [50], where they observed no statistical difference in the seroconversion rates between those couples in which the partner was receiving ART or not.

Recently, HPTN 052 RCT reported that suppression of HIV VL by early initiation of ART limits sexual transmission within HIV sero-discordant couples. In fact, 1763 couples in which one partner was HIV-1–positive and the other was HIV-1–negative were enrolled in nine countries; 54% of the subjects were from Africa, and 50% of infected partners were men. HIV-1–infected subjects with CD4 counts between 350 and 550 cells/mm^3 were randomly assigned in a 1:1 ratio to receive ART either immediately (early therapy) or after a decline in the CD4 count or the onset of HIV-1–related symptoms (deferred therapy). The primary prevention end point was linked HIV-1 transmission to HIV-1–negative partners. The primary clinical end point was the earliest occurrence of pulmonary tuberculosis, severe bacterial infection, a World Health Organization stage 4 event, or death. As of February 21, 2011, a total of 39 HIV-1 transmissions were observed (incidence rate, 1.2 per 100 person-years; 95% confidence interval [CI], 0.9 to 1.7); of these, 28 were virologically linked to the infected partner (incidence rate, 0.9 per 100 person-years, 95% CI, 0.6 to 1.3). Of the 28 linked transmissions, only 1 occurred in the early therapy group (hazard ratio, 0.04; 95% CI, 0.01 to 0.27; P<0.001). Subjects receiving early therapy had fewer treatment end points (hazard ratio, 0.59; 95% CI, 0.40 to 0.88; P = 0.01). The authors concluded that early initiation of antiretroviral therapy reduced rates of sexual transmission of HIV-1 and clinical events, indicating both personal and public health benefits from such therapy [51].

The anti-retroviral medications available have their actions at distinct stages of the viral cycle. Integrase inhibitors act before viral integration into host DNA, reverse transcriptase inhibitors and protease inhibitors act later in this cycle. Both integrase inhibitor based regimen (Stribild: co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate) and NRTI based regimen (Atripla: co-formulated emtricitabine/tenofovir DF/efavirenz) result in high rates of viral suppression and increases in CD4 cell count But patients receiving elvitegravir/cobicistat/emtricitabine/tenofovir exhibit a more rapid decline in HIV-1 RNA and a greater proportion had viral load suppressed to less than 50 copies/ml as compared to patients receiving emtricitabine/tenofovir DF/efavirenz Both these regimens have once daily dosing but faster rates of viral suppression and comparatively favorable safety profile make co-formulated

elvitegravir/cobicistat/emtricitabine/tenofovir a preferred option for initiation of treatment during acute HIV infection.

At a population level, several ecological studies reported a reduction in incidence following an increase in ART availability. An ecological study from Taiwan [48] reported a reduction of 53% in new positive HIV tests after the introduction of free access to ART; this occurred despite no change in rates of syphilis (which is used as an indicator of sexually transmitted disease transmission). Another study, based on a large cohort of MSM in San Francisco [49] showed a 60% reduction in HIV incidence that the authors attribute to availability of ART. Again in San Francisco, amongst MSM an uptake of 72% of HIV voluntary counseling test (VCT) was observed and it was followed by 95% acceptance of immediate ART for those diagnosed with HIV. This produced a reduction in 'community viral load' (i.e. the viral load of the whole community of those with diagnosed HIV) of 45% between 2004 and 2008 and an estimated 33% fall in HIV incidence between 2006 and 2008 [47]. A similar pattern was reported in British Columbia, Canada, between 1996 and 2009, where a 52% reduction in the number of new HIV diagnoses was observed against an increase of 547% in the number of patients actively receiving ART and despite a syphilis epidemic, strongly indicating increased risky behavior [52, 53]. However, earlier ecological studies investigating the effect of widespread use of ART on HIV incidence among MSM obtained contrasting results [54, 55], suggesting that any decrease in risk of HIV transmission due to ART could possibly be counterbalanced by increases in the number of unsafe sexual episodes. Particular caution needs to be taken when considering ecological studies, because they have serious limitations. In particular, ART could be not the only factor causing a reduction in HIV transmission in the region under study.

3.3 FEASIBILITY

So is it reasonable to consider that use of ART could reduce incidence of HIV to zero, and hence end the epidemic? This would require that testing coverage were 100%, ART initiation were immediate for all people diagnosed, that ART immediately reduces infectivity to zero (regardless of sexual risk behavior), and that nobody ever stops taking ART or suffers virologic failure while on ART. Below we consider how close or far away from these ideal conditions we are.

3.3.1 Testing Coverage and Frequency

One hundred percent coverage of testing is not feasible, at least not without coercion which is unacceptable. It remains unclear how many individuals in different settings have ever been tested for HIV infection and indeed how often individuals should be tested.

Among 19 countries in sub-Saharan Africa, the number of individuals aged over 15 who received HIV testing and counseling in 2008 varies from 210 per 1000 people in Botswana to two per 1000 people in Somalia [56]. It is estimated that in this region, 80% of people living with HIV are not aware of their HIV status and 90% do not know their partner's status [57]. In the UK, the rate of HIV testing, despite a dramatic increase between 1995 and 2005, remained low at 71.3 and 61.2 tests per 100 000 person-years for men and women in 2005 [58]. It is estimated that one-third of those infected with HIV in the UK are unaware of their diagnosis [59, 60]. In USA the situation is definitely better; in the last 12 months 16% of the population aged 18–64 years reported having been tested for HIV [61], but the U.S. Centers for Disease Control

and Prevention estimated that 21% of people living with HIV in USA and 27% of those in Canada are unaware of their HIV status [62].

A further indication of failure to test regularly, if at all, is the proportion of HIV diagnoses classed as 'late', that is having a CD4 cell count value of less than 350 cells/ml or AIDS within 3 months of diagnosis [63]. In Europe, the incidence of late diagnosis ranges from 15% to 45% [64]. In particular, in the UK, an estimated 32% of adults diagnosed with HIV in 2008 had CD4+ cell count less than 200 cells/ml within 3 months of diagnosis [59].

Many of those diagnosed late in the course of their disease have not previously been offered HIV testing. It has been shown in a number of studies that most newly detected cases of HIV have had contact with medical services in the last years but were not offered HIV testing [65, 66], and the same was true for late presenters [66, 67].

3.3.2 Time from HIV Diagnosis to Initiation of Antiretroviral Therapy

Immediate initiation of ART in those found to be HIV positive is fraught with difficulties, especially if, in order to increase HIV test uptake, these tests are not conducted where HIV care is delivered. At the moment no data are available on the feasibility, acceptability or deliverability of tightly linking HIV testing with treatment initiation. A RCT [68] is currently ongoing in the USA investigating the possibility of delivering this approach: the feasibility of enhanced community-level testing, and the capacity of linking HIV positive patients to care and treatment. Unfortunately these results will not be generalizable to African or any developing country context. In any case, it is important to bear in mind that although current guidelines recommend ART initiation in HIV-infected patients when CD4+ cell count reaches 350 cells/ml, worldwide only 30% of those currently in need (with 'need' defined by a CD4+ cell count < 200 cells/ml) are receiving treatment [69] and the situation is worse in many developing countries. The implementation of a policy of test-and-treat would dramatically increase the number of people starting treatment, and the source of resources to fund such initiatives is not obvious. However, the current paradigm of care is not sustainable and it will become ever more difficult to sustain without a large increase in treatment funding [70].

A further key issue is whether starting ART at higher CD4+ cell counts confers individual health benefit. At the moment this is not clear. Findings from observational studies suggest that there should be a benefit on AIDS progression and death in those who start treatment early [71, 72], but there is ongoing debate about appropriate methods for analyzing observational data and, unlike RCTs, none can be relied on to eliminate all confounding. The START trial is currently recruiting worldwide ART-naive patients with CD4+ cell counts greater than 500 cells/ml. The aim of the study is to evaluate whether immediate ART initiation is associated with reduced risk of serious AIDS-associated illness, serious non-AIDS illness and death, compared to those who defer ART initiation until CD4+ cell count reaches below 350 cells/ml [71]. This is a major component of global efforts to assess the potential impact of a test-and-treat strategy, as most strategies propose that ART be given immediately regardless of CD4 count.

3.3.3 Extent of Reduction in Infectivity with Antiretroviral Therapy and Durability of Viral Load Suppression

The potential reduction in infectivity for patients on ART has been described above, but it takes time to achieve viral load suppression and therefore a reduction in infectivity. It is estimated that 90% of patients achieve viral suppression to less than 50 copies/ml in 4 months after starting

ART, but 20% of them will at some point experience viral load rebound [73]. There is also the potential for variations in viral load on a regular basis which do not constitute failure, that is viral load 'blips' above the limit of detection in people for whom re-suppression subsequently occurs [74, 75]. In addition, failure of treatment and often large increases in viral load will go undetected and unknown by individuals until the next clinical review and viral load measurement. The impact of recognized variations of HIV-RNA in genital secretions despite undetectable serum viral load [72] is unclear. Studies have shown that ART suppresses seminal shedding of HIV [12, 75], but breakthrough genital tract HIV shedding has been observed [76]. Additionally, antiretroviral agents currently in use have different penetration into the male and female genital tracts, so can potentially lead to different rates of infectiousness [77-79]. The level of plasma HIV-RNA below which transmission does not occur (in particular in MSM) is also currently unknown. Therefore, it is necessary that patients on ART continue to be advised to avoid unprotected sex with people of unknown or negative HIV status.

3.4 RISKS

There is also a lack of information on potential negative effects of a universal test-and-treat policy, in particular the risk of increasing transmission of drug-resistant strains [80-82] induced by poor adherence to ART, the risk of sexual disinhibition [83-85] and the risk of stigmatization.

3.4.1 Drug Resistance

Although concerns have been expressed with regards to the potential risk of HIV drug resistance acquisition induced by suboptimal adherence in settings where scale-up of ART is taking place [81], recent data suggest that good adherence can be attained in resource-limited settings and in marginalized populations in developed nations [70].

This contrasts with data from some mathematical models which suggests that if antiretroviral treatment was offered to most HIV-positive individuals in South Africa eligible for treatment, drug-resistant strains could increase to up to 20% of circulating HIV strains [86]. It is likely that the risk of increased HIV-resistance caused by such a strategy has been overestimated, given that ART suppresses in most people who are adherent. In addition observational data from British Columbia has shown that the number of new cases and identified drug resistant isolates dropped when ART was extended to all intravenous drug users [53], in contrast to concerns previously expressed regarding the risk of an epidemic of drug resistant HIV. Although not an immediate concern for ART programs (relative to priorities of providing ART for those in need), in the long term, significant transmission of drug resistant virus is likely to occur if ART is used for decades without monitoring of viral load to guide switches to second line therapy [87], and this phenomenon could be more marked if ART coverage is very high. When considering the overall impact of a test-and-treat policy on transmission of drug-resistant HIV it is important to consider the incidence of infection with drug resistant virus, not the proportion of new infections with drug resistance.

3.4.2 Increased Sexual Risk Behaviors

The risk of sexual disinhibition that the wide scale use of ART could cause has been raised by some researchers [83-85, 88-91].

Most studies, but not all [92], did not find an association between use of ART and risky sexual behavior [14, 93-96] and in fact interrupting ART or low adherence were correlated with greater

risk behavior [83, 96-98]. Studies from Africa, where positive prevention was promoted, showed that 6 months and 1 year after initiation of ART risky sexual behavior was reduced [99, 100] and there was no sign of sexual disinhibition even after 2 years [93]. The concern is higher with regard to MSM. Studies showed that since 1996 there has been a substantial increase in unprotected sex among HIV-positive and HIV-negative MSM in Europe, Australia, Canada and the USA, but in the last years it seems to be leveling off [101]. The reason for this is not clear [101]; some studies showed that MSM could change to unprotected sex when perceiving less threat of HIV/AIDS since availability of highly active antiretroviral therapy [102-104] or if they believe that having suppressed viral load reduces their infectiousness [83]. In any case, a recent meta-analysis [105] investigating this issue among MSM in the USA found that being on antiretroviral therapy, having an undetectable viral load, and reporting more than 90% medication adherence were not associated with unprotected sex. Mathematical models warned that the effect on incidence of decrease in infectiousness induced by treatment could be easily counterbalanced by much more modest increases in unsafe sex or a decrease in adherence to treatment, although this will depend on exactly how low is the risk of transmission through unprotected sex from people with suppressed viral load.

3.4.3 Ethical Issues

The test-and-treat strategy is not without ethical concerns as it potentially involves a move from medical treatment for individual benefit to medical treatment for public health benefit [40]. Although the benefits of treating an individual with advanced HIV disease obviously outweigh any potential treatment-related risks, such as development of drug resistance and adverse effects of ART, the benefits to an individual diagnosed early with an intact immune system are still far from clear. Indeed, if trials were to show that early use of ART does not lead to any individual health benefit, or the toxicity and inconvenience of early ART for patients outweighs clinical benefits, then initiation of treatment at diagnosis in people with high CD4 cell count would only be justified in people well informed of the health risks who nevertheless wish to reduce their infectiousness. There is also a danger of stigmatization of groups in which HIV prevalence is high and of coercion of individuals to be tested. These issues are considerable and require due consideration.

3.4.4 Mathematical Models

Mathematical models can be useful for evaluating the potential efficacy of a strategy of test-and-treat. By definition, models are based on assumptions, upon which the validity of the inferences derived depend, but they provide an important means of explicitly relating a set of assumptions to a predicted outcome in terms of reduced HIV incidence. Models have varied in the degree of detail with which they have modeled this process.

The first mathematical simulation models produced conflicting findings regarding the effect of ART on the spread of HIV [80, 84, 85, 88-91, 106, 107]. Although most of them concluded that an increase in use of ART could lead to a decrease in HIV incidence, the likely realistic magnitude of the effect remains poorly characterized. The Granich model [108] advocating that universal voluntary HIV testing and immediate ART treatment with combined prevention interventions could potentially eradicate HIV infection has received lots of interest, but some underlying assumptions are highly controversial [109] because they assume virtually everyone could be tested and treated immediately if found HIV-positive and that accompanying prevention interventions would also be highly effective. This is not likely to be feasible [41]. Some argued [39] that a test-and-treat policy would not be the most cost-efficient strategy in some settings

and found that if it does not reach full implementation or coverage could paradoxically increase long-term ART costs [39]. Others have argued that acute infections have a major role in driving the epidemic and doubt that this strategy could diminish transmission during the acute phase [42, 109].

A recent study [43] forecast outcomes of different combinations of HIV screening and ART initiation strategies, if were implemented in Washington, District of Columbia, using a mathematical model. They showed that an intensive test-and-treat strategy (annual screening with ART initiation at diagnosis) may have a population benefit, reducing overall life-years spent with transmissible HIV infection over the next 5 years by 15%, but they concluded that such a program could not eradicate the epidemic in the District of Columbia. It is important to note that this model projects the clinical course of HIV disease but does not model new transmissions, so it does not take into account the potential reduction in risk behavior following diagnosis. Additionally, it assumes imperfect linkage and loss to follow-up among patients detected by a screening program, whereas those detected outside of the screening program are all linked to care.

3.4.5 Conclusion

We do not know

1. How effective ART that suppresses HIV-RNA in plasma will be in reducing risk of transmission on a population level. The impact of variations of HIV-RNA in genital secretions despite undetectable plasma viral load is not fully understood.

2. Whether ART has individual health benefits at higher CD4 cell counts and if not, whether people with high CD4 cell counts will accept treatment simply to reduce transmission.

3. Whether individuals will be adherent enough to ART to sustain predicted prevention benefits.

4. Whether individuals will increase their sexual risk behavior, in particular once they become aware of the reduction in infectiousness induced by virally suppressive ART.

5. Whether such a strategy would be feasible in the face of limited resources.

So even if observational studies and trials demonstrate that ART can successfully prevent transmission of infection in high-risk populations and that individuals remain highly adherent and do not experience sexual disinhibition, the critical issue remains the feasibility of such a strategy. The success of any test-and-treat strategy depends on being able to offer frequent HIV testing, on achieving a high level of acceptability in the population, on linking all detected cases to care and on having the ability to initiate ART immediately.

Cluster randomized trials that compare HIV incidence in areas that adopt a test-and-treat policy with those that do not are planned and will provide the best means of evaluating the impact of such strategies. In the meantime, models that incorporate more real life details are now needed to better understand the potential of this strategy and to assess its likely cost-effectiveness. Given that many people with HIV who are in immediate need of treatment are undiagnosed, and that a reduction in risk behavior has been observed after diagnosis, which is likely to be particularly significant for sex with longer term partners, it is already clear that a key priority for all countries should be to instigate high levels of testing, particularly in higher prevalence

groups. Whether countries should then take the next step and also have a policy of initiating ART in those with high CD4 cell count at diagnosis is yet to be established but should continue to be actively studied.

3.5 ACUTE HIV INFECTION [110]

3.5.1 The HIV-1 Transmission Event

More than 80% of adults infected with HIV-1 became infected through the exposure of mucosal surfaces to the virus; most of the remaining 20% were infected by percutaneous or intravenous inoculations [2]. The risk of infection associated with different exposure routes varies [111], but no matter what the transmission route, the timing of the appearance of viral and host markers of infection is generally uniform and follows an orderly pattern [112]. Immediately after exposure and transmission, as HIV-1 is replicating in the mucosa, submucosa, and draining lymphoreticular tissues [113, 114], the virus cannot be detected in plasma; this so-called eclipse phase generally lasts 7 to 21 days [115, 116]. Once HIV-1 RNA reaches a concentration of 1 to 5 copies per milliliter in plasma, the virus can be detected with the use of sensitive qualitative methods of nucleic acid amplification [117]; at concentrations of 50 copies per milliliter, HIV-1 can be detected by means of quantitative clinical assays used to monitor viral load [118]. The stages that define acute and early HIV-1 infection are characterized by the sequential appearance of viral markers and antibodies in the blood (Fig. 1) [112]. More sensitive, fourth-generation tests, which detect both antigens and antibodies, shrink the virus-positive–antibody-negative window by about 5 days [119]. Testing for viral RNA in plasma closes this gap by an additional 7 days.

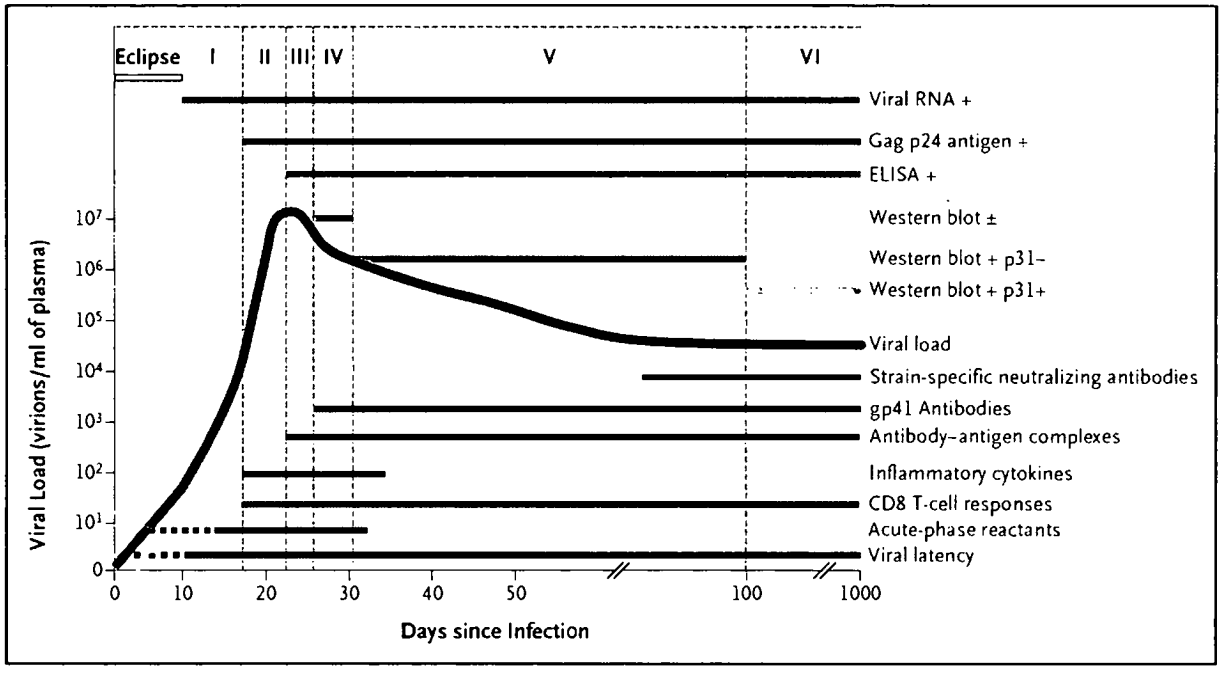

Figure 1. Natural History and Immunopathogenesis of HIV-1 Infection (from [110]).
The progression of HIV-1 infection can be depicted as six discrete stages [113] (indicated by Roman numerals). These stages are defined according to the results of standard clinical laboratory tests (listed above the curve for viral load). The stages are based on the sequential appearance in plasma of HIV-1 viral RNA; the gag p24 protein antigen; antibodies specific for recombinant HIV-1 proteins, detected with the use of an enzyme-linked immunosorbent assay (ELISA); and antibodies that bind to fixed viral proteins, including p31, detected on Western immunoblot. A plus sign indicates a positive test result, a minus sign a negative result, and a plus–minus sign a borderline-positive result. The lines below the viral-load curve show the timing of key events and immune

responses that cannot be measured with standard clinical laboratory assays, beginning with the establishment of viral latency. Acute-phase reactants include elevated levels of serum amyloid protein A. CD8 T-cell responses lead to the appearance of escape mutants concurrently with inflammatory cytokines in plasma. Immune complexes of antibodies with viral proteins, such as the HIV-1 envelope glycoprotein (gp41), precede the first appearance of free antibodies to gp41. Strain-specific antibodies to gp41 that neutralize the virus do not appear until sometime close to day 80. The portion of the line for viral latency that is dotted reflects uncertainty as to exactly when latency is first established; the dotted line for acute-phase reactants indicates that not all patients have elevated levels of reactants at this early point in the process of infection; the gray segment of the black line for viral load reflects the inability to measure very low viral loads.

The characteristic appearance in the blood of viral markers of acute HIV-1 infection belies an extremely complicated and still poorly understood series of virus–host cell interactions in the tissues [111, 120]. Given the varied routes of viral transmission — cervicovaginal, penile, rectal, oral, percutaneous, intravenous, *in utero* — and the distinctly different histologic features of these tissues, it is not surprising that several cell types are candidates for early infection. More is known about vaginal transmission than about other routes, and the study of human tissue explants [121, 122] and the Indian rhesus macaque model of vaginal transmission of the simian immunodeficiency virus (SIV) [120, 123-125] have been informative. The preponderance of evidence implicates CD4 T cells and Langerhans' cells as the first targets of the virus [121, 122], but other dendritic cells may play an important accessory role [126]. However, recent observations of mucosally transmitted strains of HIV-1 reveal that monocyte-derived macrophages are generally poor targets for infection as compared with CD4 T cells [127, 128].

Regardless of the route of viral transmission and the first cells infected, within a few days, viral replication converges on the lymphoreticular system of the gastrointestinal tract (i.e., gut associated lymphoid tissue) [129-132]. In this tissue, in both humans and macaques, the phenotype of most productively infected cells appears to be the resting CD4 T cell lacking activation markers and expressing low levels of the chemokine receptor CCR5 [123-125]. Many of these cells express $\alpha 4\beta 7$ integrin receptors and type 17 helper T–cell surface markers [133, 134]. Since these receptors are also detected on T cells harvested from the genital mucosa, they may play an important role in HIV acquisition [135]. The rapid expansion of HIV-1, first in gut-associated lymphoid tissue and then systemically [132, 136], along with a sharp rise in plasma levels of viral RNA, is clinically important because of the coincident irreversible destruction of reservoirs of helper T cells and the establishment of viral latency (defined as the silent integration of HIV-1 DNA into the genomes of resting T cells, an effect that has stymied curative treatment efforts [137, 138].

Rather than being genetically homogeneous, RNA viruses, including HIV-1, consist of complex mixtures of mutant and recombinant genomes called quasi-species. Genetic studies of the HIV-1 quasi-species in patients with chronic infection as compared with patients with acute infection have brought some clarity to the qualitative and quantitative aspects of HIV-1 transmission [115]. Figure 2 depicts the HIV-1 transmission event [115, 116, 139, 140], in which the inoculum (e.g., semen, cervicovaginal secretions, or blood) contains a complex genetic quasi-species of viruses, of which only a very small number are likely to broach mucosal barriers and establish infection. Lee and colleagues9 developed a model that allows transmitted viral genomes to be inferred from a phylogenetic analysis of the viral quasi-species that replicate in the weeks after infection. Empirical analyses based on single-genome amplification of HIV-1 RNA in plasma or HIV-1 DNA in blood lymphocytes have provided robust evidence to support this model [115, 123, 127, 140-145]. A single virion is responsible for HIV-1 transmission in approximately 80% of heterosexuals but in only about 60% of men who have sex with men and about 40% of injection-drug users [115, 127, 128, 140, 144, 145]. In injection-drug users, as many as 16 transmitted virions have been found to be responsible for productive infection [141], which would be consistent with the absence of a mucosal barrier to transmission. The phenotypes of cloned proviruses corresponding to transmitted (or founder) viruses are nearly always CD4 and

CCR5 T-cell tropic variants and exhibit neutralization-sensitivity patterns that are typical of primary viral strains. These phenotypic properties are present at the moment of transmission, when the virus encounters the first target cell; they are not the consequence of viral adaptation to the new host [115, 127, 128].

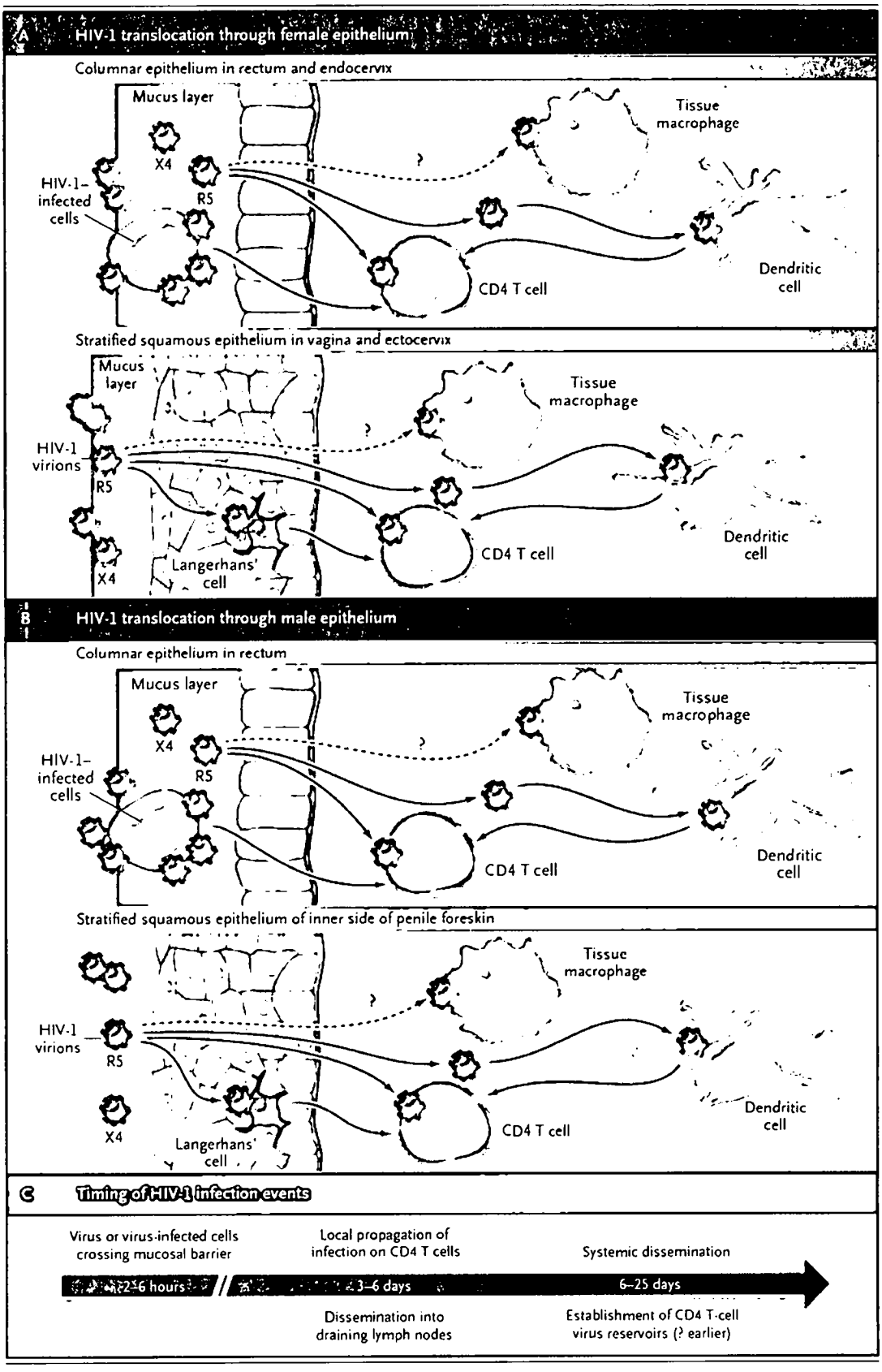
A HIV-1 translocation through female epithelium
Columnar epithelium in rectum and endocervix
Mucus layer
X4
R5
HIV-1–infected cells
Tissue macrophage
CD4 T cell
Dendritic cell
Stratified squamous epithelium in vagina and ectocervix
Mucus layer
HIV-1 virions
R5
X4
Langerhans' cell
Tissue macrophage
CD4 T cell
Dendritic cell
B HIV-1 translocation through male epithelium
Columnar epithelium in rectum
Mucus layer
X4
R5
HIV-1–infected cells
Tissue macrophage
CD4 T cell
Dendritic cell
Stratified squamous epithelium of inner side of penile foreskin
HIV-1 virions
R5
X4
Langerhans' cell
Tissue macrophage
CD4 T cell
Dendritic cell
C Timing of HIV-1 infection events
Virus or virus-infected cells crossing mucosal barrier
Local propagation of infection on CD4 T cells
Systemic dissemination
2–6 hours
3–6 days
6–25 days
Dissemination into draining lymph nodes
Establishment of CD4 T-cell virus reservoirs (? earlier)

Figure 2. Progression of HIV Transmission to Productive Clinical Infection (from [110]).
HIV-1 must traverse several tissue layers in the female vagina or rectal mucosa to come into contact with appropriate receptive cells (Panel A). The CCR5 (R5) viral strain has selective transmission advantages that remain poorly explained, and R5 variants make up the majority of transmitted and founder viruses. CXCR4 (X4) variants are transmitted only rarely. Founder viruses come into contact with Langerhans' cells or CD4 T cells in squamous epithelium; CD4 T cells can also be infected by viruses bound to submucosal dendritic cells. It is not clear whether submucosal macrophages are an initial target, since most founder viruses poorly infect macrophages in vitro. The challenge for HIV-1 transmission in the male genital tract differs somewhat from that in the vagina because of differences in anatomy, but the penile foreskin and urethra harbor critical virus-receptive cells (Panel B). Virus–cell interactions in the male submucosa are likely to be similar to those in female submucosa, with viral targets including Langerhans' cells, other submucosal dendritic cells, and CD4 cells. Removal of the foreskin through elective circumcision can prevent at least 60% of HIV-1 infections in men [114]. Although the time required for HIV-1 virions or virus-infected cells to traverse epithelial barriers is short (hours), it probably takes as long as 3 to 6 days for HIV-1 infection and propagation to occur and for the virus to spread beyond submucosal CD4 T cells (Panel C). Dissemination into draining lymph nodes and the systemic circulation rapidly follows, with establishment of the CD4 T-cell viral reservoirs. Studies in nonhuman primates of the timing of response to postexposure prophylaxis with antiretroviral drugs suggests that the time to establishment of the CD4 T-cell reservoir may be as short as 24 hours [113].

3.5.2 Detection of HIV Infection

In the absence of a high degree of clinical suspicion, the symptoms associated with acute HIV-1 infection are often too vague or nonspecific to lead to a diagnosis [146]. In the absence of antibody seroconversion, confirmation of acute infection requires detection of HIV-1 RNA or p24 antigen, but tests designed for this purpose have heretofore not been routinely available. In public health settings, a cross-sectional screening strategy that involves searching for HIV RNA in pooled, antibody-negative samples has been used to increase detection [146]. This approach has been used to detect acute HIV-1 infection, with a prevalence of 0.5 cases detected per 1000 persons tested, in North Carolina, to 4.0 cases per 1000, in San Francisco; acute infection accounted for 5 to 10% of all cases of HIV in both places.

As an alternative and more practical strategy, an enzyme-linked immunosorbent assay that can concomitantly detect viral p24 antigen and antiviral antibodies has been developed and approved for clinical use [146-148]. This test can increase the number of patients with acute HIV-1 infection whose condition is diagnosed at a time when they are most infectious to others [148]. It is anticipated that a rapid point-of-care test will also be developed for the purpose of detecting acute HIV-1 infection. The implementation of these tests across the United States in public health and commercial laboratories can be expected to dramatically increase the number of patients with acute HIV-1 infection who will require care.

3.5.3 Public Health Consequences of Acute HIV-1 Infection

The per-person probability of transmitting HIV-1 is most closely correlated with the viral burden in blood; each time the viral burden in an HIV-1–infected person increases by a factor of 10, the risk of transmission is expected to increase by a factor of 2.5 [27]. The risk of contagion from patients with acute, early infection appears to be much higher than that from patients with established infection [25], at least in part because of the high viral load and the homogeneity of viral variants clearly capable of causing infection. In the rhesus macaque SIV model, plasma from animals with acute infection is up to 750 times as infectious, on a per-virion basis, as plasma from animals with chronic infection [149]. The reduced risk of contagion from patients with chronic infection probably results from the presence of neutralizing antibodies, which are not evident in acute infection.

Mathematical models used to estimate the role of patients with acute infection in the spread of HIV-1 have produced strikingly different results depending on the population studied and the assumptions used (Fig. 3) [23, 150-160]. The epidemic phase used for modeling has been a critical determinant [161]. In communities subject to a new epidemic, early infections are held to

be responsible for a considerable share of HIV-1 transmission, since a larger proportion of infected persons have acute or early-stage disease rather than late-stage disease [162]. Sexual behavior plays an important role in rates of infection, with high rates of partner change increasing the chances of contact with a person who has acute HIV-1 infection. In a recent comprehensive study conducted in Lilongwe, Malawi, in which both behavioral and biologic data were used, 38% of cases of HIV-1 were ascribed to sexual exposure to patients in the first 5 months of infection, even though there is a long-established epidemic in Malawi. The results of the Malawi study may be most relevant to the HIV-1 pandemic in sub-Saharan Africa. The importance of acute HIV-1 infection can also be seen in studies of phylogenetically related cases. In Montreal more than half the patients with newly diagnosed early HIV-1 infection are infected with viral variants that can be linked through phylogenetic studies, which suggests the presence of clusters of transmission, perhaps from patients with acute and early infection [162].

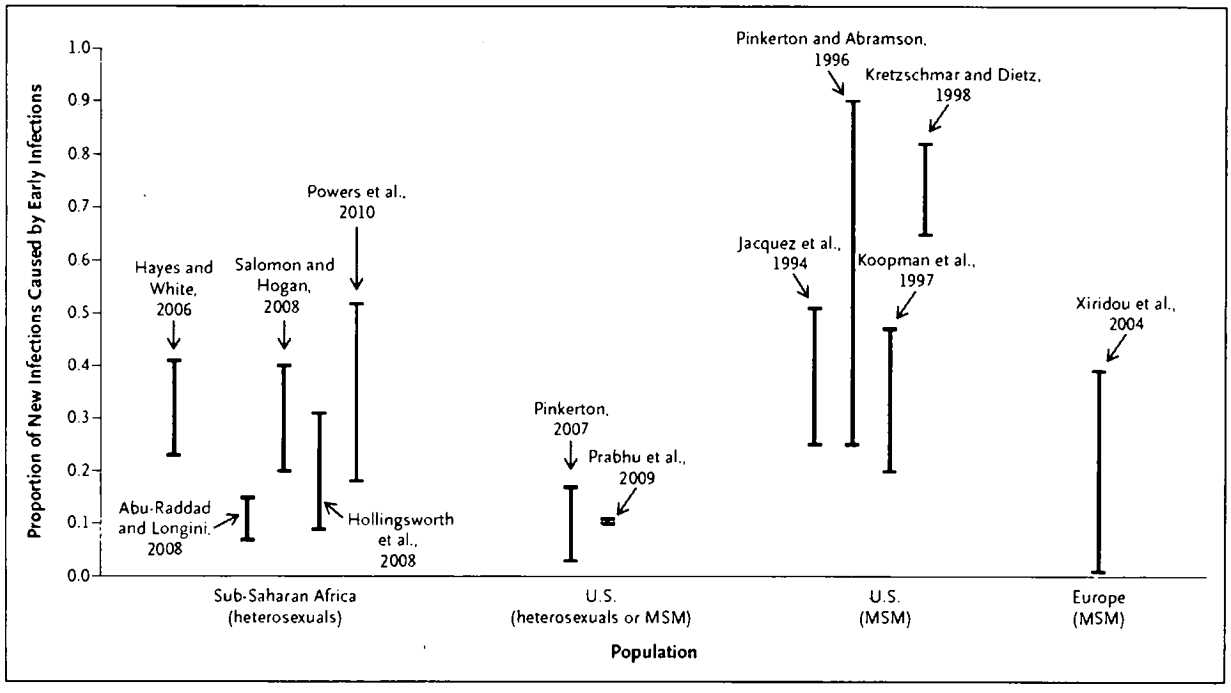

Figure 3. Role of Acute and Early HIV-1 Infection in the Spread of HIV-1, According to Population Studies in Sub-Saharan Africa, the United States, and Europe (from [110]).
Acute and early HIV-1 infection is responsible for secondary transmission of HIV-1, which is critical to the epidemic spread of the virus. A variety of models [23, 150-160] have generated widely varying estimates of the potential importance of acute and early HIV-1 infection, depending on the patient populations studied and the assumptions of the models. These models generally include people in whom the virus was detected before and during seroconversion (acute HIV-1 infection) and for several weeks thereafter (early infection) [152, 159]. The estimates reflect the proportion of all transmissions during an individual patient's entire infectious period. The extent to which this proportion corresponds to the proportion of all transmissions that occur during acute and early HIV-1 infection at the population level depends on the epidemic phase and the distribution of patterns of sexual contact in the population [154, 155, 158]. Transmission probabilities were drawn from the population category shown, but the reported estimates result from a range of hypothetical sexual-behavior variables that do not necessarily reflect a specific population [150, 155]. The range of estimates shown was extracted from the endemic-phase portion of graphs showing the proportion of new infections resulting from early HIV-1 infection over calendar time. I bars represent an estimate of the percentage of new HIV cases caused by people with acute or early HIV infection. MSM denotes men who have sex with men.

The earliest events in acute HIV-1 infection determine the future health of the individual patient and the extent of transmission in the general population. Recent studies have unraveled many of the initial immune events of acute infection. With improved diagnostic tests, greater numbers of persons with acute HIV-1 infection will come to the attention of practicing physicians and public health officials. Although considerable progress has been made in understanding the

HIV-1 transmission event, more studies are needed to develop optimal treatment and prevention strategies for people in the earliest stages of HIV-1 infection.

3.6 TREATMENT OF ACUTE AND RECENT HIV INFECTION [163]

3.6.1 Treatment for Acute HIV Infection

Clinical trials information regarding treatment of acute HIV infection is limited. Ongoing trials are addressing the question of the long-term benefit of potent treatment regimens initiated during acute infection. Previous studies, especially HPTN 052, that have demonstrated reduced risk of HIV transmission associated with ART did not include persons with acute and recent infection and were focused on discordant stable heterosexual couples in which the HIV-infected partner had an established (chronic) HIV infection. Although this study would be deferring ART for 24 weeks for some participants, the timing of this treatment initiation would still be earlier than initiation of ART in the HPTN 052 study, which initiated ART when CD4 counts were between 350 and 550 cells per mm^3. In addition, deferred study treatment would begin far earlier than standard treatment according to the Peruvian guidelines, which do not recommend initiation of treatment until CD4 counts fall below 350 cells per mm^3.

Potential benefits and risks of treating acute infection are as follows:

1. **Potential Benefits of Treating Acute Infection.** Preliminary data indicate that treatment of acute HIV infection with combination ART has a beneficial effect on laboratory markers of disease progression [164-168]. Theoretically, early intervention could decrease the severity of acute disease; alter the initial viral setpoint, which can affect disease progression rates; reduce the rate of viral mutation as a result of suppression of viral replication; preserve immune function; and reduce the risk of viral transmission during this highly infectious stage of disease. Additionally, although data are limited and the clinical relevance is unclear, the profound loss of gastrointestinal lymphoid tissue that occurs during the first weeks of infection may be mitigated by the early initiation of ART [130, 169].

2. **Potential Risks of Treating Acute HIV Infection.** The potential disadvantages of initiating therapy include exposure to ART without a known clinical benefit, which could result in drug toxicities, development of drug resistance, continuous need for therapy with strict adherence, and adverse effect on quality of life.

Some of the potential benefits associated with treatment during acute infection remain uncertain and of unknown clinical relevance, while the risks are largely consistent with those for initiating therapy in chronically infected asymptomatic patients with high CD4 counts. The health care provider and the patient should be fully aware that the rationale for therapy for acute HIV infection is based on theoretical considerations, and the potential benefits should be weighed against the potential risks. For these reasons, treatment of acute HIV infection should be considered optional at this time. Because acute or recent HIV infection is associated with a high risk of MTCT of HIV, all HIV-infected pregnant women should start a combination ARV regimen as soon as possible to prevent perinatal transmission of HIV. Following delivery, considerations regarding continuation of the ARV regimen as therapy for the mother are the same as for treatment of other non-pregnant individuals.

3.6.2 Treatment for Recent but Non-acute HIV Infection or Infection of Undetermined Duration

In addition to patients with acute HIV infection, some HIV clinicians also recommend consideration of therapy for patients in whom seroconversion has occurred within the previous 6 months. Although the initial burst of viremia among infected adults usually resolves in 2 months, rationale for treatment during the 2- to 6-month period after infection is based on the probability that virus replication in lymphoid tissue is still not maximally contained by the immune system during this time [170]. In the case of pregnancy, use of a combination ARV regimen to prevent MTCT of HIV is recommended. For non-pregnant patients the current guidelines have provided a rationale for recommending initiation of ART in ART-naive patients with CD4 count between 350 and 500 cells/mm^3 as well as a recommendation to consider therapy for those with CD4 count >500 cells/mm^3. Although these recommendations are primarily based upon data from patients with chronic infection, the potential benefit of early treatment on immune recovery and on attenuation of the pathologic effects of viremia-associated inflammation and coagulation could apply to those with early HIV infection as well. Based upon all of these considerations it is reasonable that clinicians share with patients the potential rationale for initiating ART during early HIV infection and offer treatment to those who are willing and able to commit to lifelong treatment.

3.6.3 Treatment Regimen for Acute or Recent HIV Infection

If the clinician and patient have made the decision to initiate ART for acute or recent HIV infection, the goal of therapy is to suppress plasma HIV RNA levels to below detectable levels. Data are insufficient to draw firm conclusions regarding specific drug combinations to use in acute HIV infection. Potential combinations of agents should be those used in chronic infection.

The optimal duration of therapy for patients with acute or recent HIV infection is unknown, but ongoing clinical trials may provide relevant data regarding these concerns. Difficulties inherent in determining the optimal duration and therapy composition for acute or recent infection (and the potential need for lifelong treatment) should be considered when counseling patients prior to initiation of therapy. Patients need to know that there are limited data regarding the benefits of stopping treatment, whereas strong data from studies in patients with chronic HIV infection show that stopping ART may be harmful [170].

4 STUDY DESIGN

4.1 OVERALL STUDY DESIGN

This is three-step trial. In Step 1, we will screen MSM and transgender women who are unaware of their HIV status and with evidence of high risk for acquiring HIV-1 infection, including risky sexual behavior, a partner of a newly-diagnosed person with acute or recent HIV infection or with symptoms of acute retroviral syndrome. We anticipate testing approximately 2400 MSM and transgender women until July 2015. In Step 2, high risk HIV-1 uninfected MSM and transgender women with high risk for acquiring HIV will be tested at variable intervals (during which they will receive standard HIV prevention methods) for incident HIV-1. Roughly, we anticipate testing 800 MSM and transgender women monthly for the first three months and then every other month for re-testing until July 2015. In Step 3, individuals with acute and recent HIV-1 infection detected at Step 1 or 2, or individuals who have been diagnosed with HIV

in any health clinic or laboratory authorized by the Ministry of Health, and that have had a documented negative result in the past three months, will be enrolled in a 48-week randomized, open-label clinical trial of the effects on the decay dynamics of HIV viral load in plasma, semen and rectal secretions of immediate –ARM 1- vs. deferred (by 24 weeks) –ARM 2- ART, unless ART initiation criteria are met earlier. We expect that approximately 200--250 MSM and transgender women (roughly 100-125 with acute and 100-125 with recent HIV-1 infection) will participate in the study. Those participants, who have started their participation in the study before the approval of **Amendment Letter #1** in this study protocol, will continue their participation as originally programmed. Once the **Amendment Letter #1** is approved for this study protocol, the participants who are eligible for Step 3 will be given treatment the following way: 1) those people diagnosed with **acute HIV infection** will be offered immediate ART (just at time of the enrollment visit or during the next 48 to 72 hours) for the duration of 48 weeks. 2) The people who are diagnosed with **recent HIV infection** randomized to start immediate ART or defer ART initiation until 24 weeks after HIV diagnosis, all of them followed for 48 weeks after which they will continue ART from other sources. Roughly half of these participants have been enrolled to date. These participants will receive Atripla as study drug; participants enrolled after approval of this protocol version will receive Stribild.

4.2 CRITERIA FOR OFFERING TO INITIATE TREATMENT IN STEP 3

Clinical, virologic, and immunologic criteria, as specified below, will be used to determine whether initiation of ART is indicated in subjects who have been assigned to Arm 2 (ART to be initiated at 24 weeks after HIV diagnosis). Subjects who meet these criteria for initiation of therapy will be encouraged to start ART. If the subjects agree, they will initiate therapy, which will be provided by the study while they are study participants.

Subjects in Arm 2 (ART initiation at 24 weeks after HIV study enrollment) will be offered therapy if:

1. CD4+ T cell counts fall below 350 cells/mm^3 on 2 consecutive determinations at least 4 weeks apart.

   OR

2. Participant progresses to WHO clinical stage 3 or 4 irrespective of CD4+ T cell count).

4.3 MANAGEMENT OF RESISTANCE

Plasma specimens will be stored for future testing, including HIV genotyping for antiretroviral resistance if failure to control viral load is observed during the study. Subjects with resistance to one component of the regimen may substitute another antiretroviral agent for that component (see Study Specific Procedures for recommended substitutions) but the substituted medication will be provided by the study. The substitute medication will be provided by the study while the volunteer is participating in the study.

4.4 MANAGEMENT OF SUBJECTS WITH VIROLOGIC FAILURE OR PREMATURE TREATMENT DISCONTINUATION

For subjects on immediate treatment (Arm 1), suboptimal virologic response will be defined as

the following:

1. Confirmed failure to achieve > 1 log_{10} drop in HIV-1 RNA by week 16 of first antiretroviral regimen. If at week 16 there is a < one log_{10} drop in HIV-1 RNA then the HIV-1 RNA test must be repeated as soon as possible, preferably within 2 weeks of receipt of the result.

2. Confirmed failure to achieve HIV-1 RNA level < 50 copies/mL by week 24. If at week 24 HIV-1 RNA remains ≥ 50 copies/mL, then the HIV-1 RNA test must be repeated as soon as possible, preferably within 2 weeks of receipt of the result.

3. HIV-1 RNA > 400 copies/mL on 2 consecutive measurements after having achieved HIV-1 RNA < 50 copies/mL on 2 consecutive measurements separated by at least 14 days.

If suboptimal virologic response occurs, University of Washington Virology Laboratory will perform HIV genotypying on a sample drawn before discontinuation of therapy if HIV-1 RNA ≥ 500 copies/mL, in order to determine whether or not resistance has developed and, if so, to aid in construction of a new antiretroviral regimen. The substitute medication will be provided by the study while the participant remains in the study. If the HIV genotyping reveals no evidence of of resistance and compelling arguments (i.e., substantially improved adherence or intercurrent illness to possibly explain increased viral load) the original treatment plan can be continued.. Any decision to continue medications in the setting of suboptimal virologic response must be discussed with the Study Chair/Co-Chair. Further management in terms of whether or not to begin a new or altered regimen should proceed at the discretion of the local investigator consistent with standards of care, keeping in mind that subjects are previously antiretroviral naïve and that potential treatment benefits must be weighed against the risk of further limiting antiretroviral treatment options. Subjects will be asked to remain on study to complete all study evaluations, during this time and alternative ART regimens will be provided by the Study.

4.5 MANAGEMENT OF SUBJECTS WHO DO NOT REACH 48 WEEKS IN THE ASSIGNED STUDY ARM

Subjects who do not reach 48 weeks in their assigned study arm will continue to be followed and will complete all study evaluations of the final visit. They will be treated in the analysis as detailed in Section 11.0.

5 SELECTION AND ENROLLMENT OF SUBJECTS

5.1 STEP 1

5.1.1 Description of Population

Step 1 will screen MSM and transgender women, unaware of their HIV status and with evidence of high risk for acquiring HIV-1 infection, including sexual risk behavior, partner of a newly-diagnosed person with acute or recent HIV infection, or with symptoms of acute retroviral syndrome. These men will have voluntarily visited one of our collaborating testing centers in Lima. We will also attempt to increase voluntary testing to help with our recruitment by using educational campaigns on acute HIV symptoms, community mobilization for increased frequency of testing, advertisements on radio, and recruitment of prior study participants, many

of whom in active contact with EPICENTRO, VIA LIBRE and IMPACTA (with its two clinics: San Miguel and Barranco).

5.1.2 Inclusion Criteria

Individuals will be included in the study if they meet ALL of the following criteria:

1. Male sex (at birth);
2. Eighteen years of age or older;
3. Unaware of their HIV status;
4. Ability and willingness to provide informed consent for study participation, including being tested for HIV-1; for re-testing if HIV negative (Step 2); and to consider enrollment in an immediate vs. deferred ART trial (Step 3) if diagnosed with acute or recent HIV infection after testing. People diagnosed with HIV infection during participation in Step 2 will be offered enrollment in Step 3.
5. Willingness to be re-contacted if HIV seronegative by antibody analysis but with virologic evidence of acute infection HIV-1"1" and to be linked to care;
6. Evidence of high risk for acquiring HIV-1 infection, including any one of the following:
    a. Sexual behaviors:
        i. Inconsistent condom use during anal intercourse during the last 6 months;
        ii. Anal intercourse with more than 5 male sex partners during the last 6 months;
        iii. Self-identification as a sex worker;
        iv. STI diagnosis during the last 6 months or at screening; or
        v. Sexual partner of an HIV-infected man in the last 6 months.
    b. Partner of a newly-diagnosed person with acute or recent HIV infection;
    c. Someone seeking HIV testing because of symptoms of acute retroviral syndrome;
7. Able to provide contact information, including (1) one or more phone numbers (cell and/or home) and/or a street address of residence for themselves and (2) personal contact information (ideally from two or more people) who would know their whereabouts during the study period.

1 The "1" refers to the footnote which describes the following" "**Feibig stage 1 (presence of HIV RNA only) or Feibig Stage 2 (presence of HIV RNA and antigen p24)**

8. Ambulatory performance status ≥ 80 on the Karnofsky scale.

5.1.3 Exclusion Criteria

1. Any medical, psychological/psychiatric, occupational, or other condition that, in the judgment of the investigator, would interfere with, or serve as a contraindication to, protocol adherence, assessment of safety, or a participant's ability to give informed consent;

2. Seeking HIV test behavior for routine reasons.

3. Use of estrogens (or its derivatives) or anti-androgens, administered orally or intra-muscularly, during the past three months.

5.2 STEP 2

In Step 2, high-risk MSM and transgender women with no HIV-1 infection identified in Step 1 will be asked to repeat testing every 3 months for incident HIV-1 infection while receiving standard risk reduction counseling, condoms, and management of STIs.

5.2.1 Inclusion Criterion

Individuals will be asked to participate in repeat testing if they are found to be HIV-negative in Step 1.

5.2.2 Exclusion Criterion

Any medical, psychological/psychiatric, occupational, or other condition that, in the judgment of the investigator, would interfere with, or serve as a contraindication to, protocol adherence, assessment of safety, or a participant's ability to give informed consent;

5.3 STEP 3

5.3.1 Description of Population

Step 3 will be conducted among high-risk MSM and transgender women with acute or recent HIV infection during their participation in Step 1 or Step 2 willing to be randomized to immediate vs. deferred ART, unless ART criteria are met earlier.

5.3.2 Inclusion Criteria

1. Determination of acute or recent HIV infection (either in Step 1 or Step 2), as defined by the HIV test algorithm described in Section 7.1. (Also see Appendix 1 for Study Flow Diagram and Appendix 2 for HIV categories by test results).[2]

[2] This case definition fulfills the definition of World Health Organization (WHO). According to the WHO, HIV case definition among adults and children 18 months or older is as follow:

- Positive HIV antibody testing (rapid or laboratory-based enzyme immunoassay). This is confirmed by a second HIV antibody test (rapid or laboratory-based enzyme immunoassay) relying on different antigens or of different operating characteristics;

a. Acute HIV infection:

Third-generation HIV test negative (HIV antibody negative), HIV-1 RNA test positive, and a confirmatory HIV test positive;

b. Recent HIV infection:

Third-generation HIV test positive (HIV antibody positive), HIV negative test in Step 2 in the previous 3 months, and a confirmatory HIV positive test.

Individuals who have been diagnosed with HIV in any health clinic or laboratory authorized by the Ministry of Health, and that have had a documented negative result in the past three months can also participate in Step 3.

2. No prior ART, including prior administration of pre- and post-exposure prophylaxis in the last 30 days of HIV diagnosis;

3. Laboratory values obtained within 28 days prior to study entry:

a. AST (SGOT) and ALT (SGPT) ≤ 5 x ULN;

b. Serum phosphorus > 2.0mg/dl

c. Serum creatinine < 1.4 x ULN,

d. Calculated creatinine clearance (CrCl) > 70mL/min (Cockcroft-Gault formula);

e. Serum lipase ≤ 1.5 x ULN.

4. Ability and willingness to provide written informed consent for being screened and if qualifying, to be randomized to immediate vs. deferred ART, to remain in their assigned arm and then, continue ART from other sources.

5.3.3 Exclusion Criteria

1. Presentation with WHO clinical stage 3 or 4 irrespective of CD4 count.

2. Use within 21 days prior to study entry of drugs that are highly dependent on CYP3A for clearance and for which elevated plasma concentrations are associated with life-threatening adverse events or drugs that strongly induce CYP3A and lead to lower exposure of one or more components and loss of efficacy of STRIBILD and possible resistance: alfuzosin, astemizole cisapride, ergot aklaloids and derivatives, flecainide, St. John's wort, lovastatin, simvastatin, triazolam, oral midazolam, pimozide, propafenone, rifampin, simvastatin, sildenafil terfenadine, and fluticasone-containing

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and/or;

- Positive virological test for HIV or its components (HIV-RNA or HIV-DNA or ultrasensitive HIV p24 antigen) confirmed by a second virological test obtained from a separate determination.

compounds.. Check the most recent co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (STRIBILD®) or co-formulated emtricitabine/tenofovir DF/efavirenz package inserts for other exclusionary medications. Estimated creatinine clearance below 70 mL per minute

3. Prior receipt of investigational anti-HIV vaccine.

4. Ongoing therapy with any of the following:

    a. Systemic corticosteroids. Short course less than or equal to 21 days of corticosteroids is allowed;

    b. Systemic chemotherapeutic agents;

    c. Nephrotoxic systemic agents, including aminoglycosides, amphotericin B, cidofovir, cisplatin, foscarnet, pentamidine;

    d. Immunomodulatory treatments including Interleukin-2;

    e. Investigational agents;

5. Known allergy/sensitivity or any hypersensitivity to components of study drugs or their formulations;

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

7. Serious medical or psychiatric illness that, in the opinion of the site investigator, would interfere with the ability to adhere to study requirements;

8. Chronic (HBsAg positive, anti-HBc positive, Ig M anti-HBc negative, and anti-HBs negative) or acute (HBsAg positive, anti-HBc positive, Ig M anti-HBc positive, and anti-HBs negative) hepatitis B infection.

9. Use of estrogens (or its derivatives) or anti-androgens, administered orally or intra-muscularly, during the past three months.

6 STUDY TREATMENT

6.1 STEP 1

No study treatment is offered.

6.2 STEP 2

No study treatment is offered.

6.3 STEP 3

6.3.1 Regimens, Administration, and Duration

6.3.1.1 Regimen

Those participants, who have initiated their participation in the study before the approval of **Amendment Letter #1** in this study protocol, will continue their participation as originally programmed.

Once the **Amendment Letter #1** is approved for this study protocol, the participants who are eligible for Step 3 will be given treatment the following way:

- Those people diagnosed with **acute HIV infection** will be offered immediate ART (just at time of the enrollment visit or during the next 48 to 72 hours) for the duration of 48 weeks.

  Weeks 0-48: Co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (or co-formulated emtricitabine/tenofovir DF/efavirenz) orally once daily.

Those people diagnosed with recent HIV infection will be randomized to one of the following arms:

1. Arm 1:

   Weeks 0-48: Co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (or co-formulated emtricitabine/tenofovir DF/efavirenz) orally once daily.

2. Arm 2:

   Week 0-24: No treatment unless Peruvian criteria for initiation of treatment are met.

   Week 25-48: Co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (or co-formulated emtricitabine/tenofovir DF/efavirenz) orally once daily.

6.3.1.2 Administration

Co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate will be administered as one tablet orally once daily with food. Co-formulated emtricitabine/tenofovir DF/efavirenz) will be administered as one tablet orally once daily at bedtime with or without food.

6.3.1.3 Duration

Those participants, who have started their participation in the study before the approval of Amendment Letter #1 in this study protocol, will continue their participation as originally programmed.

Once the Amendment Letter #1 is approved for this study protocol, the participants who have been diagnosed with **acute HIV infection,** and decide to participate in the study will receive treatment duration of 48 weeks.

For the people who are diagnosed with **recent HIV infection** and decide to participate, treatment will depend on which arm they are assigned to:

Arm 1 will receive study treatment for 48 weeks. Arm 2 will receive no treatment for 24 weeks and then receive 24 weeks of study treatment. However, if during the first 24 weeks of no treatment, clinical, virologic, or immunologic criteria of treatment initiation are met, subject will be offered treatment initiation. 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.

6.3.2 Product Formulation and Preparation

Elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (STRIBILD®) is co-formulated to contain 150 mg of elvitegravir, 150 mg of cobicistat, 200 mg of emtricitabine, and 300 mg of tenofovir disoproxil fumarate. in each tablet. Emtricitabine/tenofovir DF/efavirenz (Atripla™) is co-formulated to contain 200 mg of emtricitabine, 300 mg of tenofovir DF and 600 mg of efavirenz in each tablet. Each bottle contains a silica gel desiccant canister that should remain in the original container to protect the product from humidity. Dispense in the original container. Store at 25°C (77°F), excursions permitted to 15°-30°C (59°-86°F).

Co-formulated emtricitabine/tenofovir (Truvada™) contains 200 mg of emtricitabine and 300 mg of tenofovir DF in each tablet. Each bottle contains a silica gel desiccant canister that should remain in the original container to protect the product from humidity. Dispense in the original container. Store at 25°C (77°F), excursions permitted to 15°-30°C (59°-86°F).

Efavirenz of 600 mg in each tablet, will be dispensed in blister packs of 10 tablets. Store at 25 °C (77°F), excursions permitted to 15°-30°C (59°-86°F).

6.3.3 Product Supply

Co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (STRIBILD®) will be provided by Gilead Sciences, Inc while co-formulated emtricitabine/tenofovir DF/efavirez (Atripla™) will be provided by Merck & Co., Inc., under previous agreement with Gilead Sciences. Co-formulated emtricitabina/tenofovir DF (Truvada™) will be provided by Gilead Sciences. Efavirenz will be purchased locally from commercially available sources.

6.3.4 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.

6.3.5 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.

6.3.6 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 drug according to the randomization procedure below. 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.

6.3.7 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.

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.

6.3.7.1 Precautionary 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.

6.3.7.2 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.

Drugs that are Contraindicated with STRIBILD

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

Drugs that are contraindicated with Atripla

Medication Class	Prohibited Medications*
ANTIHISTAMINES	astemizole (Hismanal) terfenadine (Seldane)
ANTI-INFECTIVES	rifampin (Rifadin, Rimactane)
GASTROINTESTINAL-MOTILITY	cisapride (Propulsid)
HYPOLIPEMICS	lovastatin (Mevacor) simvastatin (Zocor)
PSYCHIATRIC MEDICATIONS	pimozide (Orap) St. John's wort (*Hypericum perforatum*)
SEDATIVES	midazolam (Versed)** triazolam (Halcion)
OTHER	alfuzosin (Uroxatral) dexamethasone dihydroergotamine (Migranal, Cafergot) eletriptan (Relpax) ergonovine (Ergotrate, Ergostat) ergotamine (Ergostat, Gotamine, Bellergal) fluticasone containing compounds (e.g., flonase, Flovent, Advair) methylergonovine (Methergine, Wigraine)

* 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.

NOTE: Subjects receiving the medications listed above who are randomized to receive immediate or deferred ART during the course of the study must agree to receive an alternative antiretroviral regimen that has been approved by the Protocol Chair.

6.3.7.3 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 this specific regimen, affects the impact of immediate and deferred ART on the decay dynamics of HIV viral load in plasma, semen and rectal secretions of individuals with acute or recent infection.

6.3.7.4 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. .

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.

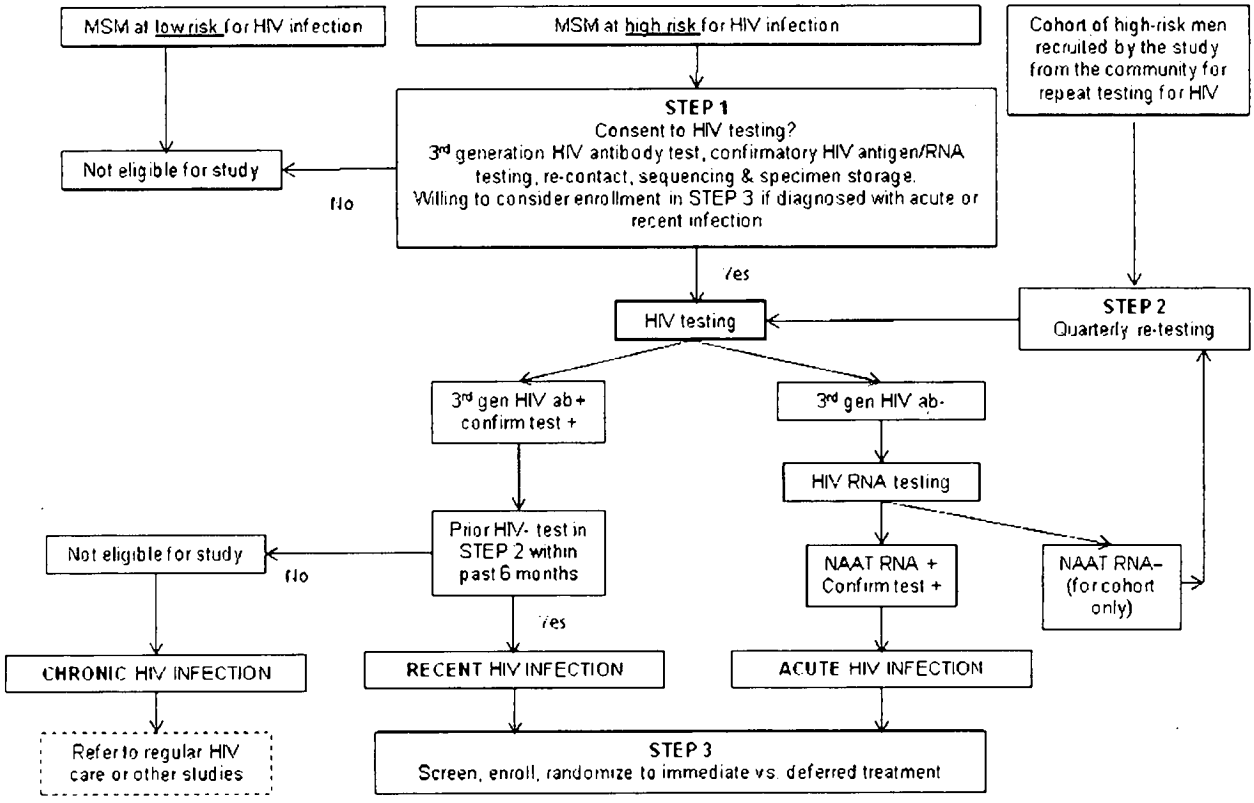
The substituted medications will be provided by the study for the duration of time that the participant remains in the study.

6.3.8 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 [171] and the Ability Likert item [172] 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 [173]. 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 [174], we also will monitor pharmacy dispensation records to produce medication possession ratios (days covered by a script/days between scripts) in approximately 3-month intervals. Pharmacy-based measures of adherence have consistently been associated with clinical outcomes in previous research [175, 176]. We will not monitor ART blood levels because a) this is not done as part of routine clinical practice, b) the tests would only refer to recent adherence rather than adherence over time, and c) it is rather expensive to conduct these tests.

7 STUDY PROCEDURES

7.1 STUDY DIAGRAM



Patient Flow Diagram

7.2 STEP 1

Written informed consent will be obtained prior to the conduct of any study procedure. Potential study participants will undergo eligibility screening (see Section 5.2.1.) by an interviewer. Eligibility will be determined based on participant responses to an interviewer-administered eligibility checklist. Study Step 1 activities will be conducted at the following sites: EPICENTRO, VIA LIBRE and IMPACTA San Miguel Clinical Research Site and IMPACTA Barranco Clinical Research Site.

7.2.1 Clinical Procedures

The following procedures will be performed at Step 1 Visit:

1. Explain the purpose of the visit and the informed consent and eligibility determination processes;

2. Determine eligibility, using Screening Eligibility Checklist (interviewer-administered);
3. Ascertain participant identity and assign Participant ID (PID) number;

4. Obtain written informed consent for willingness to consider study participation;

5. Deliver HIV and STD pre-test and risk reduction counseling; obtain written informed consents for HIV testing and stored blood for future testing. HIV and STD counseling includes encouraging, training in, and negotiation of condom use with partners and provision of lubricated condoms. Counselors will also discuss risk reduction by discussion of HIV serostatus and risks with new and existing partners, reduction of number of sexual partners, and the importance of prompt treatment for STDs;

6. Collect participant contact and locator information;

7. Administer Screening Questionnaires

8. Obtain 18.5 ml of venous blood for HIV and syphilis testing; and if acute or recent HIV infection is detected, other blood tests to evaluate overall health. These tests include liver function, Hepatitis B serology and HIV RNA;

9. Administer complete physical exam;

10. Provide results on HIV rapid test (per study HIV diagnosis algorithm –Section 7.1.) and syphilis test (diagnosis following Ministry of Health of Peru guidelines); and deliver post-test counseling;

11. Deliver counseling on potential strategies for re-contacting HIV seronegative individuals with evidence of acute infection (HIV-1 RNA positive) for further HIV testing and linkage to care;

12. Provide study site contact information and instruct the participant to contact the study site for additional information or counseling, if needed;

13. Refer for STD treatment or treat according to Ministry of Health of Peru guidelines

14. If no HIV infection is diagnosed and eligibility criteria are met (see Section 5.2.), refer to Step 2and schedule a First Visit to occur in 30 days (+/- 15 days);

15. If acute or recent HIV infection is diagnosed, refer to Step 3: Participants will be asked to come to the IMPACTA Barranco or IMPACTA San Miguel Clinical Research Site as described in the Study Specific Procedures;

16. If chronic HIV infection is diagnosed, participant is not eligible for study participation. Participant should be referred to other sources of HIV care, including standard care at no charge under Peruvian guidelines or to other studies if available;

17. Document all referrals;

18. Complete and enter all required data collection forms.

7.2.2 Laboratory Evaluations

1. HIV testing
   The following tests will be used for establishing HIV status in this protocol: Determine™ HIV-1/2 [Alere/Abbott Laboratories] (third-generation HIV test), GS HIV Combo Ag/Ab EIA [Bio-Rad] (fourth-generation HIV test) and Liat™ HIV Quant Assay [IQuum] (HIV-1 RNA test) will be used in this algorithm (see Section 7.1).

2. Sexually Transmitted Infection Testing
   Syphilis screening testing will be conducted by RPR and confirmed by MHA-TP if reactive. Syphilis diagnosis and treatment will follow Ministry of Health of Peru guidelines.

7.3 STEP 2

7.3.1 Regular Follow-Up Visit

Participants who test HIV-uninfected in Step 1 and still meet eligibility criteria (see Section 5.2.2.) will be followed in a prospective cohort study. Study Step 2 activities will be conducted at the following sites: EPICENTRO, VIA LIBRE,IMPACTA San Miguel Clinical Research Site and IMPACTA Barranco Clinical Research Site. The follow-up of participants will be every 30 days. At each follow-up visit, the following procedures will be performed:

1. Confirm participant identity and PID number;

2. Update locator information;

3. Administer Follow-up Questionnaire for assessment of sexual risk behavior and socialization venues;

4. Deliver HIV and STD pre-test and risk reduction counseling; obtain written informed consents for HIV testing.. HIV and STD counseling includes encouraging, training in, and negotiation of condom use with partners and provision of lubricated condoms. Counselors will also discuss risk reduction by discussion of HIV serostatus and risks with new and existing partners, reduction of number of sexual partners, and the importance of prompt treatment for STDs;

5. Obtain 18.5 ml of venous blood for HIV and syphilis testing (every 3 months) and if acute or recent HIV infection is detected, other blood tests to evaluate overall health. These tests include liver function, Hepatitis B serology and HIV RNA;

6. Administer targeted physical exam, if symptoms are reported;

7. Provide results on HIV rapid test (per study HIV diagnosis algorithm – Section 7.1), and syphilis test (diagnosis following Ministry of Health of Peru guidelines); and deliver post-test counseling;

8. Deliver counseling on potential strategies for re-contacting HIV seronegative individuals with evidence of acute infection (HIV-1 RNA positive) for further HIV testing and linkage

to care;

9. Provide study site contact information and instruct the participant to contact the study site for additional information or counseling, if needed;

10. Refer for STD treatment or treat according to Ministry of Health of Peru guidelines;

11. If no HIV infection diagnosis schedule a Step 2 Follow-up Visit to occur in 30 days (+/- 15 days).

12. If acute or recent HIV infection diagnosed, participants will be asked to come to the IMPACTA Barranco or IMPACTA San Miguel Clinical Research Site as described in the Study Specific Procedures;

13. If chronic HIV infection is diagnosed, participant is not eligible for inclusion in the study. Participant should be referred to other sources of HIV care, including regular care or other studies if available;

14. Document all referrals;

15. Confirm schedule for next quarterly visit;

16. Complete and enter all required data collection forms.

7.3.2 Interim Contacts and Visits

Interim contacts and visits may be conducted at participant request at any time during Step 2. Interim HIV/STD counseling and testing should be provided as needed in response to participant reports a potential high risk sexual exposure, STD symptoms, partner of a newly-diagnosed person with acute or recent HIV infection; symptoms of acute retroviral syndrome. All interim contacts and visits will be documented in participants' study records.

7.3.3 Laboratory Evaluations

Laboratory evaluation will follow what is described in Section 7.2.2.

7.4 STEP 3

7.4.1 Timing of Visits

Those participants, who have initiated their participation in the study before the approval of **Amendment Letter #1** in this study protocol, will continue their participation as originally programmed and is described here below.

Procedure	Study Visit															
		Week														
		0	1	2	4	6	8	12	16	20	24	28	32	36	40	44	48
Enrolment and sample storage informed consents		X															
Acute or recent HIV-1 counseling		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical assessment		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
TB screening		X***															
CBC		X			X		X	X			X						X
Chemistry/LFT					X		X	X			X						X
Lipid panel		X***						X			X						X
Urinalysis		X			X			X			X						X
Hepatitis B serology																	
STI testing		X						X			X						X
CD4+/CD8+ cells count		X			X			X			X			X			X
Plasma HIV-1 RNA		X	X	X	X	X	X	X	X	X	X			X			X
Semen and rectal secretion collection		X	X	X	X	X	X	X	X	X	X			X			X
Screening questionnaire - Demographics		X															
AUDIT		X															
Interviewer – Monthly questionnaire*		X			X		X	X	X	X	X	X	X	X	X	X	X
Quarterly questionnaire**		X						X			X			X			X
Pharmacy Refills					X		X	X	X	X	X	X	X	X	X	X	X
Blood volumes (ml)		60.0	10	10	21.5	10	21.5	21.5	10	10	21.5	0	0	13	0	0	21.5

***Monthly interviewer-administered questionnaire** containing: Timeline Follow Back (alcohol use and sexual behavior), Visual analog scale for ART adherence, and Medication adherence program choices

****Quarterly questionnaire** containing: Drug and alcohol use (drug use history, DAST 10, drinking expectancies); Mental health and wellbeing (RAND 12, Social support scale, Stigma scale, Stigma and disclosure, Partner notification, Intimate partner violence, 10 item CES-D, CID Mental health items on anxiety, stress & coping; Medical care (Hospitalization and ER use, Barriers to care, Access to care, Self-efficacy for ART use). *** These will be done within 72 hours.

Once the Amendment Letter #1 for this study has been approved, the participants who are eligible will enter Step 3 and they will be participate in the following way:

Procedure	Week																	
	0	1	2	4	6	8	12	16	20	24	25	26	28	32	36	40	44	48
Enrolment and sample storage informed consents	X																	
Acute or recent HIV-1 counseling	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X
Clinical assessment	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X
TB screening	X***																	
CBC	X			X		X	X			X								X
Chemistry/LFT				X		X	X			X								X
Lipid panel	X***						X			X								X
Urinalysis	X			X			X			X								X
Hepatitis B serology																		
STI testing	X						X			X								X
CD4+/CD8+ cells count	X			X			X			X					X			X
Plasma HIV-1 RNA	X	X	X	X	X	X	X	X	X	X					X			X
Semen and rectal secretion collection	X	X	X	X	X	X	X	X	X	X					X			X
Screening questionnaire - Demographics	X																	
AUDIT	X																	
Interviewer – Monthly questionnaire*	X			X		X	X	X	X	X			X	X	X	X	X	X
Quarterly questionnaire**	X						X			X					X			X
Pharmacy Refills				X		X	X	X	X	X			X	X	X	X	X	X
Hair Sample	X						X			X					X			X
Blood volumes (ml) for participants with immediate treatment	160	60	60	71.5	10	121.5	21.5	110	10	121.5	50	50	50	100	13	100	0	121.5
Blood volumes (ml) for participants with deferred treatment	230	60	60	71.5	0	201.5	21.5	190	10	201.5	50	50	50	100	13	100	0	121.5

***Monthly interviewer-administered questionnaire** containing: Timeline Follow Back (alcohol use and sexual behavior), Visual analog scale for ART adherence, and Medication adherence program choices

****Quarterly questionnaire** containing: Drug and alcohol use (drug use history, DAST 10, drinking expectancies); Mental health and wellbeing (RAND 12, Social support scale, Stigma scale, Stigma and disclosure, Partner notification, Intimate partner violence, 10 item CES-D, CID Mental health items on anxiety, stress & coping; Medical care (Hospitalization and ER use, Barriers to care, Access to care, Self-efficacy for ART use). *** These will be done within 72 hours.

7.4.2 Timing of Evaluations

7.4.2.1 Entry Evaluations (Week 0 Visit)

Subjects diagnosed with acute HIV infection must begin treatment within 72 hours after randomization, and preferably within 24 hours.Subjects randomized to Arm 1 must begin treatment within 72 hours after randomization, and preferably within 24 hours.

7.4.2.2 On-Study Evaluations

On-study evaluations will occur according to the schedule of events as specified in Section 7.4.1. On-study evaluations will occur according to the schedule of events as specified in Section 7.4.1. according to whether the participant was enrolled before or after the approval of Amendment 1, of this protocol. A study visit will be considered missed if it is not completed before half of the time between the current and next study visit has elapsed.

. Study visit windows will be +/- 5 days for scheduled study visits.

7.4.2.3 Evaluations for Subjects Randomized to Arm 1 Who Do Not Start Study Treatment

Subjects with recent HIV infection randomized to Arm 1 (immediate treatment) who do not start study treatment will be followed according to the originally specified schedule of evaluations.

7.4.2.4 Evaluations for Subjects Who Start Treatment without Meeting Eligibility Criteria

Subjects with recent HIV infection randomized to Arm 2 (deferred treatment to be started at 24 weeks after HIV-1 diagnosis) who elect to start treatment despite not meeting criteria for treatment initiation will be invited to continue to be followed according to the originally specified schedule of evaluations. Study medication will not be provided to these subjects. The ART treatment will not be provided by the study in this case.

7.4.2.5 Premature Treatment Discontinuation Evaluation Provided by the Study

Subjects who voluntarily discontinue treatment provided by the study will be advised to continue anti-retroviral treatment with public or private sources. The study investigators will take necessary steps to ensure that the subjects continue ART. Counseling will be provided with a special emphasis on safety, and later efficacy options to use in the future if the participant decides to discontinue ART provided by the study and not continue with any other. All the participants will be invited to continue with the follow-up evaluations according to the originally specified "Timing of Visits".

7.4.2.6 Premature Study Discontinuation Evaluations and Voluntary Treatment Provided by the Study

Subjects who elect to withdraw from participation in the study prior to completion of the study will have all final evaluations performed for the end of the study as described in visit for Week 48.

7.4.3 Definitions of Evaluations and Special Instructions

7.4.3.1 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. Details of acute HIV seroconversion signs and symptoms and HIV risk behaviors should be included. Any allergies to any medications and their formulations must be documented.

7.4.3.2 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.

7.4.3.3 Concomitant Medications

All concomitant medications taken since the last report must be recorded in the source documents and CRFs and must include start dates and stop dates.

7.4.3.4 Study Treatment Modifications

All modifications to study drug(s) including initial doses, subject-initiated and/or protocol-mandated interruptions, modifications, and permanent discontinuation of treatment must be recorded at each visit in the source documents and CRFs.

7.4.3.5 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. 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

7. Tuberculosis screening
   Potential participants will be taken to a nearby clinic for TB screening (sputum acid fast bacilli stain and chest X-ray). Chest X-rays will be offered as part of the study procedures. Tuberculosis prophylaxis will be provided to participants according to Ministry of Health of Peru guidelines.

8. Leukapheresis procedure
   A subset of SABES? 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 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 (baseline, 24 and 48 weeks).

9. 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.

7.4.3.6 Laboratory Evaluations

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 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. All values for total HDL and LDL cholesterol, and triglycerides should be recorded on the CRFs at baseline and every 12 weeks, regardless of toxicity grade.

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
   Subjects should be fasting for at least 8 hours when possible. Triglycerides, total cholesterol, HDL, LDL;

4. Urinalysis
   Dipstick, and microscopic exam if dipstick positive;

5. Hepatitis B serology
   Potential participants will be tested for hepatitis B infection through HBsAg, anti-HBc and anti-HBs. The interpretation of the results will be based in the US Center for Diseases Control and Prevention Hepatitis B Serology Interpretation (available at http://www.U.S. Center for Disease Control and Prevention.gov/hepatitis/HBV/HBVfaq.htm#general).

   Individuals with chronic or acute hepatitis B infection will not be eligible to participate. Hepatitis B susceptible participants will be offered hepatitis B vaccination according Ministry of Health of Peruvian guidelines.

6. 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. Further confirmation with a nucleic acid amplification test for *N. gonorrhoeae* and *C. trachomatis* will follow if a diagnosis of urethritis is made.

7.4.3.7 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.

7.4.3.8 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 for the visits 0, 12, 24, 36, and 48 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.

The plasma samples for RNA HIV-1 determination from visits 1, 2, 4, 6, 8, 16, and 20 will not be processed in real time. Since these determinations are exlusively research purposes, the samples will be processed at a later time.

2. Phylogenetic Analyses
   HIV-1 sequencing and phylogenetic analysis will be performed at The University of Washington Molecular Genetics Laboratory

7.4.3.9 Genotype

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

7.4.3.10 Semen and Rectal Secretions

Semen and rectal secretion specimens will occur according to the schedule of events as specified in Section 7.4.1. Samples will be initially processed and stored as stated in the Protocol Study Specific Procedures. HIV-1 viral load in semen and rectal secretions will be tested at the University of Washington Retrovirology Laboratory at the end of the study.

7.4.3.11 Stored Plasma

At all-time points for which an HIV-1 RNA level is required the remaining plasma after the HIV-1 RNA assay is performed will be stored.

7.4.3.12 ***Hair.*** This section has been created to describe the following:

The collection of a snip of hair (approximately 80 or 120 individual hairs) will be obtained from the scalp of the participants using a pair of scissors, as specified in section 7.4.1. If it is not possible to obtain hair from the scalp, then hair from face, extremities or pubic region may be obtained, also using a pair of scissors. The hair samples will be analyzed in ethyl glucuronide (marker for chronic alcohol consumption)[3] and a commercially available kit will be used for the analysis of cocaine, marijuana, phencyclidine, amphetamines, and opioid consumption.

7.4.3.12 Hair. As a result of the above change, the following foot note has been added:

3 Drug Testing: A white paper of The American Society of Addiction Medicine. 2013. http://www.asam.org/docs/default-source/public-policy-statements/drug-testing-a-white-paper-byasam.pdf

7.4.3.13 Questionnaires

Interviews will either be administered by interviewers (monthly) or self-administered by participants (quarterly).

The AUDIT will be administered at the first visit only, along with a short questionnaire, including demographics, health literacy, and another short questionnaire.

A questionnaire (roughly 60 minutes) will be administered at the first study visit and then quarterly. It will contain several modules as follows:

1. Drug and alcohol use (drug use history, DAST 10, drinking expectancies);
2. Mental health and well being (RAND 12, Social support scale, Stigma scale, Stigma and disclosure, Partner notification, Intimate partner violence, 10 item CES-D, CID 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).

In addition, there will be a monthly interviewer-administered questionnaire containing the following items: Timeline Follow Back (alcohol use and sexual behavior), Visual analog scale for ART adherence, and Medication adherence program choices.

The Timeline Follow Back questionnaire includes questions on sexual partners. We will collect anonymous interview data about partners (including number of partners, type of partner [main, casual, anonymous], *etc.*). We will also counsel men on the need to inform recent sexual partners that they may have been exposed to HIV. Scripts have been developed to guide this process. In brief, all participants will be provided with intensive one-on-one partner notification counseling with a trained member of the staff. First party partner notification (notification of partners by the index HIV-infected MSM and transgender women) will be encouraged and supported by use of a printed referral card. Third party notification (notification by the provider that a person may have been exposed to HIV) will be offered as an option in situations where the participant does not want to or does not feel comfortable notifying their partner directly. All third party notifications will be anonymous (*i.e.*, will not reveal participants' names or other identifying information). Any partners who wish to be tested for HIV will be offered free HIV testing at IMPACTA Barranco or IMPACTA San Miguel Clinical Research Site (the study sites).

8 WARNINGS AND PRECAUTIONS

8.1 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 elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate or co-formulated emtricitabine/tenofovir DF/efavirenz 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).

8.2 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 elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate or co-formulated emtricitabine/tenofovir DF/efavirenz is not indicated for the treatment of chronic HBV infection and the safety and efficacy of co-formulated emtricitabine/tenofovir DF/efavirenz have 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 elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate or co-formulated emtricitabine/tenofovir DF/efavirenz and are co-infected with HIV and HBV.

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

8.3 PSYCHIATRIC SYMPTOMS

Serious psychiatric adverse experiences have been reported in patients treated with efavirenz. In controlled trials of 1008 patients treated with regimens containing efavirenz for a mean of 2.1 years and 635 patients treated with control regimens for a mean of 1.5 years, the frequency of specific serious psychiatric events among patients who received efavirenz or control regimens, respectively, were: severe depression (2.4%, 0.9%), suicidal ideation (0.7%, 0.3%), nonfatal suicide attempts (0.5%, 0%), aggressive behavior (0.4%, 0.5%), paranoid reactions (0.4%, 0.3%), and manic reactions (0.2%, 0.3%). When psychiatric symptoms similar to those noted above were combined and evaluated as a group in a multifactorial analysis of data from Study AI266006 (006), treatment with efavirenz was associated with an increase in the occurrence of these selected psychiatric symptoms. Other factors associated with an increase in the occurrence of these psychiatric symptoms were history of injection drug use, psychiatric history, and receipt of psychiatric medication at study entry; similar associations were observed in both the efavirenz and control treatment groups. In Study 006, onset of new serious psychiatric symptoms occurred throughout the study for both efavirenz-treated and control-treated patients. One percent of efavirenz-treated patients discontinued or interrupted treatment because of one or more of these selected psychiatric symptoms. There have also been occasional post-marketing reports of death by suicide, delusions, and psychosis-like behavior; although a causal relationship to the use of efavirenz cannot be determined from these reports.

Patients with serious psychiatric adverse experiences should seek immediate medical evaluation to assess the possibility that the symptoms may be related to the use of efavirenz, and if so, to determine whether the risks of continued therapy outweigh the benefits

8.4 NERVOUS SYSTEM SYMPTOMS

Fifty-three percent of patients receiving efavirenz in controlled trials reported central nervous system symptoms compared to 25% of patients receiving control regimens. These symptoms included dizziness (28.1%), insomnia (16.3%), impaired concentration (8.3%), somnolence (7.0%), abnormal dreams (6.2%), and hallucinations (1.2%). Other reported symptoms were euphoria, confusion, agitation, amnesia, stupor, abnormal thinking, and depersonalization. The majority of these symptoms were mild-moderate (50.7%); symptoms were severe in 2.0% of patients. Overall, 2.1% of patients discontinued therapy as a result. These symptoms usually begin during the first or second day of therapy and generally resolve after the first 2–4 weeks of therapy. After 4 weeks of therapy, the prevalence of nervous system symptoms of at least moderate severity ranged from 5% to 9% in patients treated with regimens containing efavirenz and from 3% to 5% in patients treated with a control regimen. Patients should be informed that these common symptoms were likely to improve with continued therapy and were not predictive of subsequent onset of the less frequent psychiatric symptoms. Dosing at bedtime may improve the tolerability of these nervous system symptoms.

Analysis of long-term data from Study 006, (median follow-up 180 weeks, 102 weeks, and 76 weeks for patients treated with efavirenz + zidovudine + lamivudine, efavirenz +indinavir, and indinavir + zidovudine + lamivudine, respectively) showed that, beyond 24 weeks of therapy, the incidences of new-onset nervous system symptoms among efavirenz-treated patients were generally similar to those in the indinavir-containing control arm. Patients receiving co-formulated emtricitabine/tenofovir DF/efavirenz should be alerted to the potential for additive central nervous system effects when co-formulated emtricitabine/tenofovir DF/efavirenz is used concomitantly with alcohol or psychoactive drugs.

Patients who experience central nervous system symptoms such as dizziness, impaired concentration, and/or drowsiness should avoid potentially hazardous tasks such as driving or operating machinery.

8.5 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 it's formulations. Estimated creatinine clearance, urine glucose and urine protein should be documented in all patients prior to initiating therapy. Initiation of co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate in patients with estimated creatinine clearance below 70 mL per minute is not recommended.

Co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate and co-formulated emtricitabine/tenofovir DF/efavirenz 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 elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate therapy in all patients. Additionally, serum phosphorus should be measured in patients at risk for renal impairment.

Although cobicistat (a component of STRIBILD) 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 emtricitabine and tenofovir DF are primarily excreted by the kidney. Co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate and co-formulated emtricitabine/tenofovir DF/efavirenz 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.

8.6 SKIN RASH

In controlled clinical trials, 26% (266/1008) of patients treated with 600 mg efavirenz experienced new-onset skin rash compared with 17% (111/635) of patients treated in control groups. Rash associated with blistering, moist desquamation, or ulceration occurred in 0.9% (9/1008) of patients treated with efavirenz. The incidence of Grade 4 rash (e.g., erythema multiforme, Stevens-Johnson syndrome) in patients treated with efavirenz in all studies and expanded access was 0.1%. Rashes are usually mild-to-moderate maculopapular skin eruptions that occur within the first 2 weeks of initiating therapy with efavirenz (median time to onset of rash in adults was 11 days) and, in most patients continuing therapy with efavirenz, rash resolves within 1 month (median duration, 16 days). The discontinuation rate for rash in clinical trials was 1.7% (17/1008). Co-formulated emtricitabine/tenofovir DF/efavirenz can be reinitiated in patients interrupting therapy because of rash.

Co-formulated emtricitabine/tenofovir DF/efavirenz should be discontinued in patients developing severe rash associated with blistering, desquamation, mucosal involvement, or fever. Appropriate antihistamines and/or corticosteroids may improve the tolerability and hasten the resolution of rash.

Experience with efavirenz in patients who discontinued other antiretroviral agents of the NNRTI class is limited. Nineteen patients who discontinued nevirapine because of rash have been treated with efavirenz. Nine of these patients developed mild-to-moderate rash while receiving therapy with efavirenz, and two of these patients discontinued because of rash.

8.7 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 emtricitabine/tenofovir DF/efavirenz 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 emtricitabine/tenofovir DF/efavirenz to these patients.

8.8 BONE EFFECTS OF TENOFOVIR DF

Bone Mineral Density:
In clinical trials in HIV-1 infected adults, tenofovir DF (a component of STRIBILD) was associated with slightly greater decreases in bone mineral density (BMD) and increases in biochemical markers of bone metabolism, suggesting increased bone turnover relative to comparators. Serum parathyroid hormone levels and 1.25 Vitamin D levels were also higher in subjects receiving tenofovir DF.
The effects of tenofovir DF-associated changes in BMD and biochemical markers on long-term bone health and future fracture risk are unknown. Assessment of BMD should be considered for HIV-1 infected patients who have a history of pathologic bone fracture or other risk factors for osteoporosis or bone loss. Although the effect of supplementation with calcium and vitamin D was not studied, such supplementation may be beneficial in all patients. If bone abnormalities are suspected, then appropriate consultation should be obtained.

Mineralization Defects:
Cases of osteomalacia associated with proximal renal tubulopathy, manifested as bone pain or pain in extremities and which may contribute to fractures, have been reported in association with the use of tenofovir DF. Arthralgias and muscle pain or weakness have also been reported in cases of proximal renal tubulopathy. Hypophosphatemia and osteomalacia secondary to proximal renal tubulopathy should be considered in patients at risk of renal dysfunction who present with persistent or worsening bone or muscle symptoms while receiving products containing tenofovir DF.

8.9 CONVULSIONS

Convulsions have been observed in patients receiving efavirenz, generally in the presence of known medical history of seizures. Caution must be taken in any patient with a history of seizures.

Patients who are receiving concomitant anticonvulsant medications primarily metabolized by the liver, such as phenytoin and phenobarbital, may require periodic monitoring of plasma levels.

8.10 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.

8.11 IMMUNE RECONSTITUTION SYNDROME

Immune reconstitution syndrome has been reported in patients treated with combination antiretroviral therapy,.. 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.

8.12 CO-ADMINISTRATION WITH RELATED DRUGS

Co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate is indicated for use as a complete regimen for the treatment of HIV-1 infection and co-administration with other antiretroviral products is not recommended.
Co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate is not recommended for coadministration with the following:

- 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)

Related drugs not for co-administration with co-formulated emtricitabine/tenofovir DF/efavirenz include EMTRIVA (emtricitabine), VIREAD (tenofovir DF), TRUVADA (emtricitabine/tenofovir DF), and SUSTIVA (efavirenz), which contain the same active components as the co-formulation offered in this trial. Due to similarities between emtricitabine and lamivudine, other drugs that should not co-administered include those containing lamivudine, like COMBIVIR®, EPIVIR™, EPIVIR-HBV™, EPZICOM™, or TRIZIVIR™.

9 TOXICITY MANAGEMENT

Toxicity management guidelines take into consideration the fact that the subjects are started on their first antiretroviral drug regimen very early in the course of their HIV infection when long-term clinical benefits of antiretrovirals are unproven. Study treatment may be interrupted or discontinued at the discretion of the investigator and according to the severity of the adverse experience. However, investigators are reminded that the purpose of this study is to look at the effects of early treatment in general, not the effects of this particular regimen. Attempts should be made to continue antiretroviral treatment uninterrupted if possible. Thus, substitutions for

toxicity are allowed and would be preferable to the discontinuation or interruption of the entire regimen, if safety considerations permit. Alternate regimens will be provided for participants by the study for the duration of time that the participant remains in the study.
There will be no mandated drug interruptions or discontinuations for any Grade 1 or 2 toxicity, except for elevated creatinine. Subjects with symptomatic toxicity Grade $\leq$ 2 may continue to receive study treatment at the discretion of the investigator. Any allowed medication may be used to treat subjects for symptomatic toxicities, at the discretion of the investigator, except excluded medications as listed in section 5.3.3.

9.1 PROCEDURES FOR MODIFICATIONS FOR SPECIFIC SYMPTOMS

9.1.1 Rash

9.1.1.1 Grade 1 or 2 Rash

Study treatment should continue without interruption and the rash may be treated symptomatically with permitted antipyretic or antihistamine medications, but should be monitored closely by the local investigator.

9.1.1.2 Grade 3 Rash

All study treatment should be interrupted until the toxicity grade returns to Grade $\leq$ 1, after which time study medications can be resumed or alternative antiretroviral medications may be initiated at the investigator's discretion upon consultation with the Protocol Chair. If Grade $\geq$ 3 rash persists for more than 4 weeks, or if Grade 3 rash recurs, then the offending agent should be permanently discontinued.

9.1.1.3 Grade 4 Rash

All study treatment should be interrupted for any Grade 4 rash or evidence of a Stevens-Johnson syndrome. Any resumption of study treatment or alternative antiretroviral medications should only be done upon consultation with the Protocol Chair.

9.1.2 Nausea and Vomiting

9.1.2.1 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.

9.1.2.2 Grade $\geq$ 3

Subjects with Grade $\geq$ 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 $\geq$ 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.

9.1.3 Diarrhea

9.1.3.1 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.

9.1.3.2 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.

9.1.4 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.

9.2 TOXICITY MANAGEMENT FOR LABORATORY ABNORMALITIES

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.

9.2.1 Increases in Values for Liver Function Tests

9.2.1.1 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.

9.2.1.2 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 ≤ 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 ≥ 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.

9.2.2 Lipase

9.2.2.1 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 ≥ 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.

9.2.2.2 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.

9.2.3 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 subjects with a nonfasting triglyceride level ≥ 1200 mg/dL, a fasting triglyceride level and a serum amylase and lipase must be obtained. 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.

9.2.4 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.

9.2.5 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.

9.2.6 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.

9.3 FOR 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.

10 CRITERIA FOR DISCONTINUATION OF THE TREATMENT PROVIDED BY THE STUDY

10.1 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.

10.2 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.

11 STATISTICAL CONSIDERATIONS

The overall aim of this project is to model seek-test-treat-retain strategies that specifically intervene to reduce HIV transmission through reductions in HIV-1 viral load and sexual risk behavior among individuals with early HIV infection, including both acute and recent infections.

11.1 OBJECTIVES

11.1.1 Primary Objectives

1. To estimate the impact of immediate and deferred (6 months after diagnosis) ART on the decay dynamics of HIV viral load in plasma, semen and rectal secretions from individuals with early HIV infection;

2. To investigate the impact of drug and alcohol use on HIV transmission by examining and modeling the role of MSM and transgender women with substance abuse disorders in transmission clusters identified through partner tracing and phylogenetic analysis.

11.1.2 Secondary Objectives

1. To determine HIV incidence and associated risk factors among high-risk HIV-1 uninfected MSM and transgender women who participate in a program of frequent routine HIV testing.

2. To attempt to identify transmission clusters in phylogenetic trees constructed using early viral specimens from study participants with acute or recent HIV infection.

3. To determine other characteristics (self-reported sexual risk behavior, partner characteristics, use of entertainment venues, and method of referral for HIV testing) in MSM and transgender women within clusters compared to those outside of clusters.

4. To assess the feasibility and acceptability of re-contacting HIV seronegative individuals with evidence of acute infection (HIV-1 RNA positive) for further HIV testing and linkage to care.

5. To evaluate the frequency of HIV testing among recent sexual partners of participants diagnosed with early HIV-1 infection, comparing strategies such as partner tracing and notification, and general and targeted community outreach.

6. To evaluate retention in care, including adherence to ART and study procedures, among individuals with early HIV-1 infection receiving immediate and deferred ART.

11.2 ENDPOINTS

11.2.1 Primary Endpoints

1. To quantify HIV viral load in plasma, semen and rectal secretions in individuals with early HIV infection in immediate versus deferred treatment groups.

2. To identify transmission clusters through phylogenetic analysis among participants with early HIV infection receiving immediate and deferred ART; Construction of models to assess the potential population impact of a seek-test-treat-retain strategy.

11.2.2 Secondary Endpoints

1. To identify associated risk factors for incident HIV-1 infection.

2. To identify sequence clusters using phylogenetic analysis among participants with early HIV infection.

3. To compare self-reported sexual risk behavior, partner characteristics, entertainment venue attendance, and method of referral in MSM and transgender women within clusters to those outside of clusters.

4. To determine the proportion of individuals with evidence of acute infection (HIV-1 RNA-positive) who are successfully contacted for confirmatory HIV testing and are then linked to care.

5. To determine the proportion of newly identified HIV-positive men willing to name at least one recent sexual partner, and the proportion of named partners subsequently contacted and tested.

6. To determine the proportion of men retained in care, including adherence to ART and study procedures, among individuals with early HIV-1 infection receiving immediate and deferred ART.

11.3 SAMPLE SIZE AND ACCRUAL

Our goal is to enroll approximately 75-90 men with AHI and 75-90 with RHI in three years, with a target of enrolling 25-50 men with AHI and 25-50 men with RHI per year, or approximately 2-4 men in each group per month. We expect that another 50 men will be identified with acute or recent HIV-1 infection who will either not meet all inclusion requirements or who will not consent to enrollment in the study. Specimens for sequencing will be collected from all men.

Samples for sequencing will be obtained from all identified HIV+ partners of the 200 men with acute/recent infection (we anticipate that not all men will consent to partner tracing). Our initial sequencing efforts will focus on the 100 acutely infected men (75 enrolled plus 25 non-enrolled in immediate/deferred ART study) and their HIV+ partners (especially those with acute or recent HIV infection), testing a total of 400 samples. The first available sample will be sequenced with 454/pyrosequencing and analyzed as described below.

Semen HIV VL will be measured in samples from 60 (30 acute/30 recent) men in the immediate ART group and 60 (30 acute/30 recent) men in the deferred treatment group using established procedures in The University of Washington Virology Laboratory [177, 178]. We will seek separate funding to analyze additional samples, including rectal secretions.

11.4 MONITORING

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

11.5 ANALYSES

11.5.1 Viral Phylogenetic Analysis

Viral phylogenetic analysis will be conducted to investigate the occurrence of clustered transmission during AHI as evidenced by detection of highly related sequences among men with acute infection [162, 179, 180] and their recent partners identified through contact tracing. An algorithm for identifying HIV transmission linkages among partnerships has recently been developed by our collaborator Dr. Mullins, who analyzed sequences using phylogenetic techniques and developed a Bayesian algorithm to evaluate the probability of linkage using genetic distance measurements [181]. The same approach will be used here and applied for the first time to identifying transmission clusters.

Next-generation deep sequencing using the 454/Pyrosequencing platform will be used to generate envelope gene sequences from each study participant using plasma from the first available time point following infection. Deep sequencing provides us with the opportunity to identify relatively low-frequency variants in each infection. This is important because we have found that in a significant fraction of the cases, divergent, low-frequency variants are often involved in transmission to a new host [181]. To provide context for the identification of linked transmissions, we will prepare two HIV-1 envelope gene sequence databases corresponding to the same genetic region as Lima. The first dataset will contain all available Peruvian HIV-1 sequences from the Los Alamos National Laboratory HIV Sequence Database (HIVDB) (http://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html), as well as subtype B sequences from other countries in South America. This will enable us to determine the expected spread of genetic relationships from individuals without epidemiologic linkage. Second, a "linked" database of corresponding HIV-1 envelope gene sequences from 147 known transmission pairs [181] will be generated to enable us to determine the expected spread of genetic relationships from individuals with known epidemiologic linkage. HIV-1 linkage will be assigned by requiring the following: 1) pair derived HIV-1 env sequences from two or more individuals form monophyletic clusters (i.e., originating from the same terminal node) in maximum-likelihood phylogenetic trees that include all available sequences from unrelated individuals and 2) the pairwise genetic distances are required to be associated with a Bayesian posterior probability >50%. Pairs of sequences that meet these two requirements are tentatively classified as linked. In this manner, a phylogenetic tree containing clustered and unclustered sequences will be constructed for all acutely infected index cases and their HIV+ recent sexual contacts. These clusters are due to high-level transmission which occurs early after infection, before sequence divergence. (If funding allows or additional funding is available, specimens from men with recent infection and their partners will be sequenced and added to this analysis.)

11.5.2 Effect of HIV Seek and Test Strategies and Impact of Substance Use on Clustered Transmission:

First, initial analysis will assess process measures evaluating, for example, the % HIV infections in MSM and transgender women that are detected during AHI compared to our preliminary data from 2007-2008 and rates in historical controls [182], and the proportion of MSM and transgender women with AHI diagnosed by point-of-care vs. lab-based assays who are successfully linked to care.

Second, summary statistics on the inferred phylogenic tree will be computed, including the number of clusters identified, the percentage of MSM and transgender women in the cluster, the number of infections in each cluster, and the maximum window periods for onward transmission within each cluster. To evaluate the impact of partner services we employ on finding transmission clusters, we will also compare the percentage of MSM and transgender women in the cluster to that percentage seen in the literature using other approaches, e.g., 49.4% in [162], 34% in [183]. McNemar's test will be conducted to evaluate the agreement between being in the clusters based on partner tracing and being in the clusters based on phylogeny.

Third, we will compare the sexual behaviors, and use of alcohol or drugs such as cocaine between MSM and transgender women clustered by phylogeny and MSM and transgender women not clustered by phylogeny (using 2 set of trees: MSM and transgender women with acute/recent infection with or without partners). Our analysis of the impact of substance use on clustered transmission will include all MSM and transgender women with acute/recent infection and their partners. For example, we anticipate that roughly 5% of index cases with AHI will use

cocaine and roughly 25% will have AUD based on a recent study of high risk MSM and transgender women in Lima [184]– this analysis will assess whether either cocaine use or AUD are enriched among men in transmission clusters. For outcomes measured at a single time point, we will use Chi-squared tests, Fisher's exact tests or Mann-Whitney U tests, as appropriate. For outcomes measured longitudinally, we will use the generalized estimating equation approach to account for correlations among repeated measures.

Fourth, baseline questionnaire data on substance use and sexual behavior will also be compared between MSM and transgender women with acute and recent HIV infections after stratifying on AUD. Both univariate comparison and multiple regression to explore risk factors that are associated with acute/recent infection will be conducted.

11.5.3 The Impact of Immediate and Early (6 Months after Diagnosis) Antiretroviral Therapy on the Decay Dynamics of HIV Viral Load

For men with acute/recent infection plasma VL will be measured at 0, 1, 2, 4, 6, 8, 12, 16, 24, 36 and 48 weeks. Semen specimens and rectal secretions will be collected from all men with acute/recent infection at 0, 1, 2, 4, 6, 8, 12, 16, 24, 36 and 48 weeks and stored. Semen HIV VL will be measured in these samples from 60 (30 acute/30 recent) ART-treated men and 60 (30 acute/30 recent) untreated men using established procedures in the University of Washington Virology Laboratory [177, 178]. This sample size is adequate to detect a 1.0-1.5 $\log_{10}$ change in VL associated with ART based on the following: semen viral load peaks about 30 days after infection, with mean 4.5 and standard deviation 1.6 ($\log_{10}$ copies/ml) [185]. We compute the power of a two-sample t-test comparing two arms (ART vs. no ART) to detect significant difference with type I error 5%. With 30/group we have 66% power to detect a 1.0 $\log_{10}$ change and 94% power to detect a 1.5 $\log_{10}$ change based on a single viral load measure at peak per subject. The power would be greater if we use any summary measure of individual's multiple viral loads, e.g., mean across different time points.) We will seek separate funding to analyze additional samples, including rectal secretions. VL in semen and rectal secretions will be more informative for modeling the potential impact of ART on infectivity during this period than plasma VL as these 3 measures are only modestly related [177, 178].

The viral load trajectory will be plotted for each subject and for plasma, semen and rectal secretions separately. The mean trajectory and its confidence interval will be computed stratified by randomized arm and by acute or recent infection. The first-order rate of decay, the area under the curve, the peak viral load, and the lowest viral load will be computed for each subject and compared across arms. Multiple testing adjustment will be applied to control type I error.

11.5.4 Modeling the Expected Intervention Impact at Population Level

Data for model development will come from several sources. We will use data from the study testing centers to estimate the percentage of total HIV infections detected during AHI and the distribution of testing intervals among MSM and transgender women attending participating VCT centers. We will use results of the formative research to modify the baseline questionnaire compiled by Dr. Altice from multiple validated surveys such as the Addiction Severity Index, Brief Symptom Inventory, etc. and use it to collect extensive data from all enrolled participants on sexual risk behaviors, drug and alcohol use, adherence to ART, social support, mental health and psychiatric status. Data will be collected from men with Acute and recent HIV infection (and consenting HIV+ partners) with or without alcohol use disorder. Data on sexual networks will be obtained by partner tracing and phylogenetic analysis. Clinical (CD4+ cell count, HIV viral load)

and adherence data (pill count, recall) will be used for analysis and incorporated into models as appropriate. Data will be managed at the Data Management Center (DMC) at the IMPACTA Barranco Clinical Research site, with experienced staff that participated in multiple NIH-funded studies. The DMC will be responsible for forms development, data entry, database management, adverse event reporting, specimen tracking, report generation, quality control and data security.

Mathematical modeling will address the potential impact of two strategies to reduce onward HIV transmission – treatment of AUD, and detection and treatment for AHI. A sequence of deterministic models will be developed to simulate HIV transmission in MSM and transgender women populations (with and without interventions) and to evaluate the public-health impact of the increased detection and treatment of acute and recent (within 3-6 months) HIV infections on the HIV epidemic among MSM and transgender women. Individuals in these models will be divided in classes with respect to serostatus, stage of HIV infection, and with respect to their ART status (treated/untreated). Men will be additionally stratified by alcohol and drug dependency, level of sexual activity, and behavioral risk. The rates at which men acquire HIV, i.e., forces of infections, will be expressed as functions of the number of partners per susceptible person, the number of sex acts per partnership, and the transmission probability per sex act for partners from different classes using standard binomial risk formulas. We expect to encounter 300 seropositive men per year with about 10% of them with recent infections. In addition, we expect to identify 30-45 acutely infected men per year. Infectivity estimation will use plasma and semen VL data from ART treated and untreated men with acute/recent HIV infection as well as set-point plasma VL from 200 men with established HIV infection (100 with/ 100 without AUD). These data will allow us to construct the initial epidemic distribution of the MSM and transgender women population and to dynamically calibrate the models.

12 DATA COLLECTION, MONITORING AND ADVERSE EXPERIENCE REPORTING

12.1 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.

12.2 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 of 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.

12.3 CLINICAL ADVERSE EVENTS

Clinical AEs will be evaluated by the study physicians. Grade 3 and 4 clinical AEs will be referred to Study Medical Director (on-site physician responsible for medical decision-making) 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 physician responsible for medical decision-making, by phone at all times.

12.4 LABORATORY ADVERSE EVENTS

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 Study Medical Director 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.

12.5 DISCONTINUATION OF STUDY AGENT DUE TO AN ADVERSE EVENT

The Study Medical Director 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.

12.6 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 be obliged to 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.

12.7 ASSESSMENT OF 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(or co-formulated emtricitabine/tenofovir DF/efavirenz) 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?.

12.8 FOLLOW-UP ON 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.

12.9 REVIEW OF ADVERSE EVENTS

Summaries of combined AE reports will be reviewed and discussed by the Protocol Team and clinic medical staff. All AEs will be tabulated and meeting minutes will be documented. These reports will be provided to the NIDA Program Official after review by the Protocol Chair.

12.10 EXPEDITED ADVERSE EVENTS REPORTING TO NATIONAL INSTITUTE ON 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.

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

The study drug on which the relatedness assessment is to be based is co-formulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (or co-formulated emtricitabine/tenofovir DF/efavirenz) and reported to the NIDA Program Official.

12.10.2 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.

12.10.3 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 bases, i.e., from publicly available information.

12.11 CONTACT FOR QUESTIONS ABOUT EXPEDITED ADVERSE EVENTS REPORTING

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

12.11.1 Assuring Reporting of Suspension of 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.

13 HUMAN SUBJECTS PROTECTION

13.1 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.

13.2 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.

13.3 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.

14 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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