Protocol
Effects of mu-opiate receptor engagement on microbial translocation and residual immune activation in HIV-infected, ART suppressed opioid use disorder patients initiating medication-assisted treatment

Acronym	AMOHI-1 (Anti-retroviral therapy, Medications for opioid use disorder, Opioids and HIV infection – Study 1)
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Regulatory sponsor	University of Pennsylvania (David S. Metzger, PhD)
NIH Grant Number	NIDA R01 DA048728-01
Investigational Product	methadone, buprenorphine/naloxone, extended-release naltrexone
IRB Number	833720
ClinicalTrials.gov Number	Not yet applied

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List of Abbreviations

ART: Antiretroviral therapy

AE: Adverse event

BUP/NX: Buprenorphine/naloxone

MAT: Medication for opiate use disorder-Assisted Treatment

MET: Methadone

MOR: Mu Opiate Receptor

MOUD: Medication for opiate use disorder

SAE: Serious Adverse Event

UDS: Urine Drug Screen

XR-NTX: Extended-Release Naltrexone

Study Summary

Title	Effects of mu-opiate receptor engagement on microbial translocation and residual immune activation in HIV-infected, ART suppressed opioid use disorder patients initiating medication-assisted treatment
Short Title	AMOHI-1
IRB Number	833720
Protocol Number	NIDA R01 DA048728-01
Phase	Phase II
Methodology	3-arm randomized-controlled study
Study Duration	60 months
Study Center(s)	Go Vap clinic, Ho Chi Minh city, Vietnam
Objectives	Primary: • To compare plasma sCD14 concentration in HIV-positive opiate use disorder individuals who receive cART and methadone, buprenorphine/naloxone or XR-naltrexone over 48 months Secondary: • To evaluate the change in markers of cellular immune activation and senescence, systemic inflammation and bacterial translocation • To evaluate the effectiveness of the treatment administered towards a) reducing/suppressing opiate injection behavior and promoting adherence to the treatment schedule and b) suppressing HIV replication
Number of Subjects	225 (75 per arm)
Main Inclusion and Exclusion Criteria	Inclusion criteria: • Age: male or female subjects 18- 65 years of age • Meet DSM-5 criteria for moderate to severe opiate use disorder (as determined by DSM-5 checklist) • Documented HIV-1 infection with CD4<350 cells/ μL and VL> 10,000 copies/mL Exclusion criteria • Meet DSM-5 criteria for any other substance use disorder (except nicotine) • Already engage in opiate medication treatment at baseline (methadone, buprenorphine, buprenorphine/naloxone, naltrexone) • Advanced liver disease (FibroScan® METAVIR score F3-F4, liver elasticity >10kPa)

Investigational Product (drug, biologic, device, etc.) **For Device include the planned use** **For Drug, food, cosmetic, etc. include the dose, route of administration and dose regiment**	Methadone: daily dosing, oral Buprenorphine/naloxone: daily dosing and thrice-weekly after stabilization, sublingual Extended-release naltrexone (Vivitrol®): monthly, depot injection
Duration of administration (if applicable)	12 months
Reference therapy	None (not intend to evaluate the efficacy of one treatment versus another but to evaluate change in markers of inflammation along a course of treatment)
Statistical Methodology	The primary outcome of interest is the change in sCD14 over 48 weeks following ART. This marker will be assessed over 7 consecutive time points (baseline, weeks 4, 8, 12, 24, 36, and 48). The primary analyses for this longitudinal data will use the mixed model regression model (MER) with restricted maximum likelihood
Safety Evaluations	• Liver function test • Adverse events and serious adverse events including hospitalization and overdose
Data and Safety Monitoring Plan	The study will be monitored by the PIs and co-investigators, University of Pennsylvania Institutional Review Board, Ho Chi Minh City CDC Institutional Review Board, Ho Chi Minh Pasteur Institutional Review Board, as well as by regulatory committees at the University of Pennsylvania (i.e., IRBs, OHR) and Center for Studies of Addiction Data Safety Monitoring Board (Penn DSMB). During the course of the study, safety and data quality monitoring will be performed on an ongoing basis by the Principal Investigators and the study staff. Dr. Charles O'Brien (Center for Studies of Addiction, University of Pennsylvania) will serve as the medical monitor for the study.

This document is a clinical research protocol. This study will be conducted in full accordance all applicable University of Pennsylvania Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46. All episodes of noncompliance will be documented.

1 Introduction

1.1 Background and Relevant Literature

Chronic opiate use results in a range of adverse effects including increased susceptibility to infections and systemic immune activation. Opioid-induced over-expression of TOLL-like receptors (TLR) 2 and 4 in the gut mucosa leads to alterations in the epithelial tight junctions; in rodents this depends on the activation of myosin light chain kinase (MLCK), and results in bacterial translocation to the liver. Binding of opioids to cells of the enteric nervous system EMS plexa leads to decreased gut motility, resulting in chronic constipation and accumulation of bacterial products. Chronic opioid exposure results in changes in the gut microbiota (increase in Gram+ bacteria of the phylum *Firmicutes*, previously associated with systemic inflammation), which is TLR-2-dependent and may be related to acidification of the bile secretion [1-4]. Chronic opioid use has also been shown to effect innate immune functions, such as a reduction in NK cell activity through central dopaminergic signaling [5]. Thus, lack of immune clearance may also contribute to systemic immune activation.

Three medications for opiate use disorder (MOUD) have been approved and showed efficacy in the treatment of opiate use disorder, all interacting with the mu opiate receptor (MOR) either as a full (methadone, MET) or partial (buprenorphine, BUP) agonists, or as an antagonist (naltrexone, NTX). The effect of persistent engagement of MOR on immune activation by MET remains controversial. Early reports indicated that production of IL-2, IL-10, IFN-γ, but not IL-6, was normalized in subjects on MET. However, recent work evidenced high serum levels of IL-1β, IL-6, and IL-8 in MET users [6, 7]. On the other hand, MOR antagonist Naltrexone (and particularly its extended-release formulation, XR-NTX) has demonstrated efficacy in supporting abstinence in individuals with opioid and alcohol use disorders [8] [see also guidelines of the American Society of Addiction Medicine [9]], including HIV-1 infected individuals transitioning from the criminal justice system to the community [10]. In vitro, NTX exerted a direct anti-HIV effect by reversing the opioid-induced overexpression of the co-receptor CCR5 on peripheral blood mononuclear cell [11]. Clinical use of NTX-based MOUD was also shown to improve HIV suppression on ART over placebo [12], mostly due to improved ART adherence, and was not associated with liver toxicity [13], even in the presence of HCV co-infection [14].

Worldwide, the HIV epidemic has involved three main transmission modalities: sexual transmission, vertical perinatal transmission, and by needle sharing during IV drug injections. Regarding the latter, Vietnam has a large population (currently about 271,000 individuals) of people who inject drugs (PWIDs), 85% of which inject opiates [15]. In 2014, approximately two-thirds of the reported 15,000 new HIV infections in Vietnam were attributable to injection drug use [15]. Since 2008, Vietnam has instituted outpatient MET-based MOUD programs. Oral NTX is also currently available at selected addiction clinics (including the proposed study site: Thu Duc Addiction Treatment Center, Ho Chi Minh City, http://tuvancainghien.gov.vn). However, it is estimated that only 20-22% of individuals with opioid use disorder have access to MAT [16, 17]. Vietnam has also implemented a massive scale up of ART, but in 2015 only a third of HIV-positive individuals were on ART. The most common risk factor for late ART initiation is active drug-injecting behavior [18]. Integration of MOUD within HIV services has resulted in improved ART outcomes (e.g., enrollment, adherence, retention, and viral suppression) in multiple settings [19, 20], including Vietnam. However, there is less evidence to support that MAT decreases mortality on ART [21]. Vietnamese PWIDs have very high rates of co-infections with HCV (ranged from 89.8% and 98.5% [21, 22]), HBV (ranged from 20-40%, [23]), and tuberculosis (209 cases per 100,000 inhabitants, [24]). HIV-HCV co-infection in PWIDs has been estimated as high as 98.5% [25], but unlike TB infection, it does not appear to significantly increase mortality in these settings [26, 27].
This proposal takes advantage of an established collaboration with a large urban clinic providing ART and MOUD to HIV-infected PWIDs concurrently. Since November 2013, our group has implemented an integrated opioid use disorder and HIV-1 treatment program at Go Vap Clinic, Ho Chi Minh City, Vietnam, sponsored by a NIDA Grant (R01-DA033671). This program enrolled 448 heroin users with an average age of 32.4 years (SD= 5.2) and living with family (81%). The prevalence of HIV in the cohort was 34.2% (n=153). Participants received integrated treatment with either MET (n=268) or buprenorphine/naloxone (n=180) [28].

1.2 Name and Description of the Investigational Product

The participants will be randomized in three arms:

- Methadone (MET) is a full opiate agonist of the mu opioid receptor. Methadone has been used for decades to treat people who have opioid use disorder. When taken as prescribed, it is safe and effective. Methadone hydrochloride oral concentrate available in Vietnam is provided by Mallinckrodt PLC.
- Buprenorphine/naloxone (BUP/NX) is a partial mu opiate receptor agonist and a kappa receptor antagonist. It has been approved for clinical use since October 2002 by the Food and Drug Administration (FDA). BUP/NX, in combination with counseling and behavioral therapies, provide a whole-patient approach to the treatment of opioid use disorder. When taken as prescribed, BUP/NX is safe and effective.
- Extended-release naltrexone (XR-NTX) is a full mu opiate receptor antagonist. Naltrexone is a medication approved by the Food and Drug Administration (FDA) to treat opiate use disorder. The injectable extended-release form of the drug (Vivitrol) is administered at 380 mg intramuscular once a month. Naltrexone can be prescribed by any health care provider who is licensed to prescribe medications. To reduce the risk of precipitated withdrawal, patients are warned to abstain from illegal opioids and opioid medication for a minimum of 7-10 days before starting naltrexone. XR-NTX is not yet available in Vietnam. For the study, we are using Vivitrol® provided by Alkermes, Inc.
- All participants will receive combined antiretroviral therapy (cART) for HIV.
- All participants will receive counseling sessions for 48 weeks.

1.2.1 Clinical Data to Date

The extent, change, and immunopathogenesis of residual immune activation in HIV-infected individuals receiving suppressive ART have important clinical repercussions as elevated expression of sCD14 (positively correlated to microbial translocation) has been repeatedly reported to be associated with higher mortality and adverse metabolic outcomes in ART-suppressed individuals with chronic HIV-1 infection [29-32]. In addition, T cell activation and particularly the expression of CD38 and HLA-DR on CD8+ T cells is associated with a lack of $CD4^+$ T cell recovery on ART, and a loss of 35 $CD4^+$ T cells/μl for each 5% increase in activation [33]. Thus, identifying the potential benefits of choosing MOR antagonists over MET after ART may better inform addiction treatment choices and public health policy.

We have completed a pilot study assessing the expression of immune activation markers and soluble microbial translocation markers in a cross-sectional convenience cohort of HIV-1-infected PWIDs (and control, non-opioid injecting patients) receiving ART and MOUD at the Jonathan Lax Clinic/Philadelphia FIGHT (Philadelphia, PA). Within the limitations of a small convenience cohort, our pilot data strongly supports the hypothesis that in PWIDs receiving (or initiating) ART, continued MOR engagement through MET may result in impaired immune reconstitution outcomes as compared with NTX.

2 Study Objectives

The overall objective of this study is to provide clinical evidence on the detrimental link between kinetics and characteristics of immune reconstitution (microbial translocation, residual immune activation, retained HIV expression) in HIV-1 infected people who inject drugs (PWIDs) due to a sustained interaction with the μ-opioid receptor (MOR) while on antiretroviral therapy (ART).

2.1 Primary Objective

- To compare plasma sCD14 concentration in HIV-positive opiate use disorder individuals who receive cART and methadone, buprenorphine/naloxone or XR-naltrexone over 48 months. To this end, we will compare long-term changes (48 weeks) in a cohort of PWID with chronic HIV infection initiating ART and initiating either MET, BUP/NX or XR-NTX along with behavioral drug and risk counseling (BDRC) sessions.

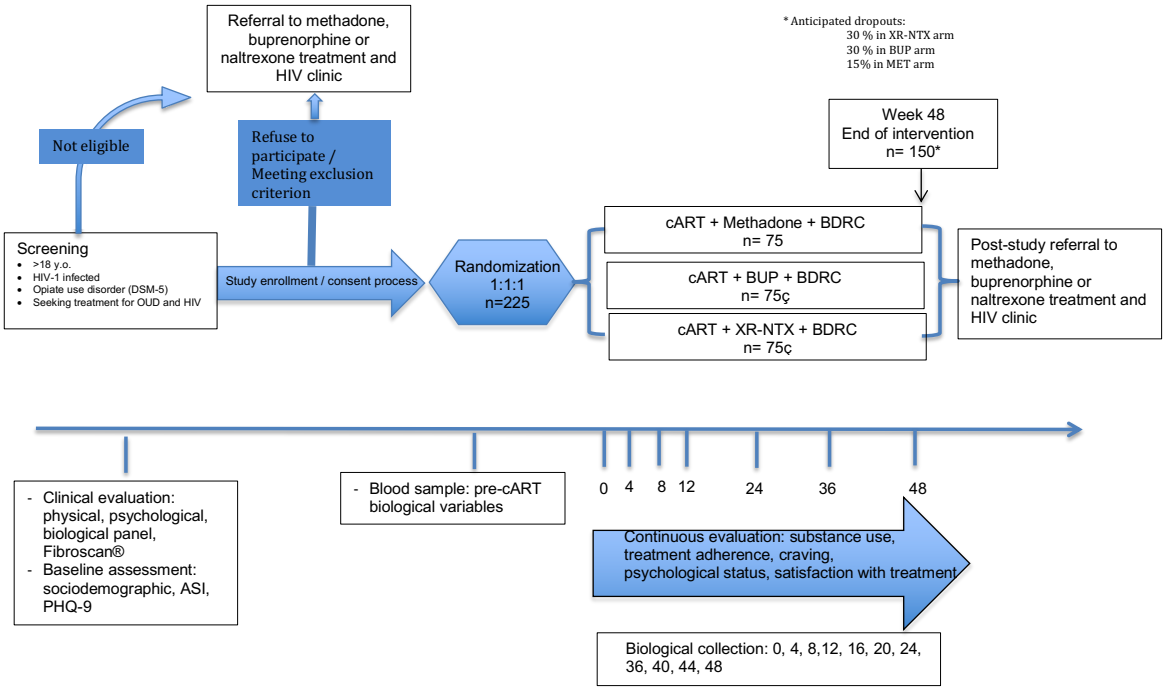
2.2 Secondary Objectives

- To evaluate the change in markers of cellular immune activation and senescence, systemic inflammation and bacterial translocation.

- To evaluate the effectiveness of the treatment administered towards a) reducing/suppressing opiate injection behavior and promoting adherence to the treatment schedule and b) suppressing HIV replication
    - The effect of the intervention on CD4, adherence to ART, acceptability of MOUD, as well as retention in care
    - The changes in HIV replication and latency measures (i.e., characterization of viral RNA and DNA in PBMC, persistence of cells latently infected with potentially replicative HIV in circulation)

3 Investigational Plan

3.1 General Design



3.1.1 Screening Phase

Participants will be recruited as they enter treatment at the Go Vap HIV and methadone clinic. Clinical staff at the HIV and addiction clinic will identify potential subjects and refer them to the research assistant (RA). The RA will describe the study and those who express interest will be referred to the study medical supervisor who will explain it in detail, answer questions, review the consent to screen, and have the subject sign it after confirming that he/she understands it. Tests to confirm eligibility for cART will be done on site. For those eligible, informed consent will be obtained in writing by the study medical investigator from all study participants prior to any procedure. Study personnel will first introduce potential participants to the study and explain the study procedures. A written informed consent form (ICF) translated in Vietnamese, the content of which will be approved by the local Ethics Committee according to local regulations, will also be provided to the candidates, who will be allowed time to read it and discuss it with the study personnel. ICFs will clearly state that participation in the research is completely voluntary and that there will be no consequence to the participant for declining participation or absences from study procedures. Candidates who consent to participate will sign and date the form; signed ICFs will be locked-stored at the Go Vap site according to local regulation and GCP directives.

3.1.2 Study Intervention Phase

All the participants will be evaluated at the clinic by the study medical director who will perform physical examinations and review laboratory work, document current opiate use and DSM-5 opiate use disorders, perform a clinical interview to rule out psychiatric disorders that exclude participation, review the ICF, and

answer any remaining questions. A Fibroscan® will be performed for each potential participant, and those with a METAVIR score F3-F4 will be excluded from the study and will be referred to hepatitis C specialist for care.

Following completion of informed consent procedures, a RA will complete baseline assessments, and randomize the participant to his/her medication condition. The study medical director will then coordinate the care at the clinic for cART (with HIV specialist) and methadone, buprenorphine or long-acting naltrexone treatment. A treatment plan with scheduled visits will be delivered to the participant.

Treatment initiation

- Methadone arm

The induction on methadone will follow the Vietnamese guidelines. Basically, the dosing will commence with 20 to 30 mg daily; thereafter, dose increases will be made following review of the participant's response to their initial methadone dose. Assessments will review side effects, symptoms of withdrawal (suggesting not enough methadone) or intoxication (suggesting too much methadone or other drug use), ongoing cravings, and substance use. Generally, the doses are increased 5 to 10 mg every three to five days. The dose will be gradually increased until a maintenance dose is reached, which consists of cessation (or marked reduction) of opioid use, alleviation of cravings and opioid withdrawal features between doses, whilst minimizing methadone side effects. Daily directly observed methadone dosage will be delivered along with weekly for 12 weeks, and then monthly counseling sessions up to 48-week follow-up.

- Buprenorphine/naloxone arm

The induction on buprenorphine/naloxone will follow the Vietnamese guidelines. Buprenorphine/naloxone tablets are administered sublingually, placed under the tongue until they are dissolved. For doses requiring the use of more than two tablets, participants are instructed to either place all the tablets at once or alternatively (if they cannot fit in more than two tablets comfortably) place two tablets at a time under the tongue. Either way, the participant should continue to hold the tablet under the tongue until they dissolve; swallowing the tablet reduces the bioavailability of the drug.

To aid clinicians in determining the appropriate dose during the first two-to-three days, the study will use the Clinical Opiate Withdrawal Scale (COWS), an instrument measuring validated items of physical signs or symptoms of withdrawal, such as gooseflesh, vomiting, and sweating, to objectively assess withdrawal in both treatment arms [34]. The total score gives an index of the participant's withdrawal intensity and can be administered over time to track changes and adjust dosing. On day one, the clinician will wait until the participant shows at least mild opiate withdrawal by monitoring with the COWS. If the score indicates at least mild withdrawal (the higher the score the more severe the withdrawal), the clinician will give 4 mg for the first dose. COWS will be repeated in one hour; patients will receive further dosing as described below. Dosing will begin with a titration over a period of two to three days under supervision in the Go Vap clinic using the COWS as described above. On the first day of treatment, patients will initially receive a 4 mg dose of buprenorphine/Naloxone (expressed as the amount of buprenorphine) to be taken sublingually. Most participants will begin with a total first day's dosage of 8 mg. On Day 2, up to 16 mg may be given. Up to 32 mg may be given on Day 3 and thereafter until three-times-weekly dosing begins. The induction strategy is primarily dependent on three factors: 1) time since last opiate use; 2) type of opiate (e.g., long or short-acting) used; and 3) degree of physical dependence. Therefore, each dosing schedule will be tailored to the individual participant.

Individuals will come to the study site daily for direct observation of dosing until they have stabilized (for up to three weeks). After induction and stabilization, participants will be asked to come to the site for dosing three-times-weekly. The target dosage schedule for individuals whose daily dose was 16 to 24 mg/day is expected to be 32/32/48 mg administered on a three-times-weekly schedule (e.g., M/W/F); this is also the maximum three-times-weekly dosage. On rare occasions, for individuals who require more than 24 mg/day (i.e., 26, 28, 30, or 32 mg/day), it is unlikely that the 32/32/48 mg dosage schedule will be adequate. For those individuals, as well as for others who received 24 mg or less per day but for whom the 32/32/48 mg three-times- weekly schedule is not adequate, dosing may be continued on a daily basis through Week 52 of the study, with take-home doses administered for those days on which in-clinic dosing is not possible (e.g., 32 mg on M/Tu/W/Th/F/Sat with a take-home 32 mg dose on Sun).

Daily or thrice-weekly buprenorphine dosage will be delivered along with weekly for 12 weeks and then monthly counseling sessions up to 48-week follow-up.

- Extended-release naltrexone (XR-NTX) arm

The participants assigned to naltrexone arm will undergo supervised 2-week opioid inpatient detoxification at the Thu Duc Addiction Treatment Center (under the supervision of Dr. Do). The detoxification program follows Vietnamese guidelines (http://tuvancainghien.gov.vn). Adjunctive medications might also be used depending of the severity of the withdrawal symptoms, including benzodiazepines for anxiety and restlessness (e.g., oxazepam); low doses of sedating antidepressants (e.g., doxepin, trazodone) or zolpidem for insomnia; antiemetics for GI distress (e.g., prochlorperazine, ondansetron); and nonsteroidal anti-inflammatories for withdrawal-related aches. Withdrawal symptoms will peak at 24-48 hours after heroin cessation. Three counseling sessions will be scheduled during the detoxification, and participants will have access to counselors via telephone between sessions. The first XR-NTX will occur at day 12-14 of the detoxification with at least one day stay at the clinic before discharge and beginning of the outpatient follow-up. Before injecting XR-NTX and as a confirmation of detox period, participants will receive a naloxone (fast acting opiate blocker) challenge. The challenge will be performed by slowly administering intramuscularly 0.8-mg naloxone; if no withdrawal symptoms occur within 20 minutes, the absence of physiologic reaction is confirmed. Participants who experience withdrawal symptoms following the naloxone challenge will be treated symptomatically, observed until symptoms resolve, and will be offered counseling and invited to 2 days of extended detoxification for a repeat naloxone challenge. Failure to pass the challenge on 3 occasions disqualifies a patient from study participation, and a negative outcome will be considered an endpoint in our intention to treat analysis. If a participant elects not to be part of the study for whatever reason, he/she will be strongly encouraged to receive opiate use disorder treatment and will be referred to methadone treatment or oral naltrexone treatment. The XR-NTX will be injected monthly by Dr. Trung Nguyen and Dr. Do Mahn who will receive training and supervision from Dr. George Woody (who has extensive experience in XR-NTX treatment in the USA and internationally).

- cART (three arms)

cART will follow Vietnamese guidelines for HIV treatment. We will look to coordinate to start methadone or XR-naltrexone within 2 weeks of cART, but starting cART will take priority over methadone/naltrexone in order to avoid losing patients due to the logistic issues in scheduling appointments. To minimize the chance of developing resistance attributable to poor adherence from relapse in patients who happen to start cART before methadone or XR-naltrexone, we will start each treatment within 2 weeks of each other. Participants who change their mind or are found ineligible at any point will be referred to the usual addiction treatment and encouraged to continue with the HIV treatment program. Declining enrollment in the study will not affect eligibility for cART.

- Counseling (three arms)

The counseling session is modeled after the Behavioral Drug and Risk Counseling (BDRC) designed to address both addiction and HIV related behaviors [35, 36]. This model is rooted in cognitive behavioral therapy. Each session will assess the need for intervention in six areas of functioning: 1) adherence to treatments for Substance Use Disorder, HIV, TB; 2) continued drug use and related drug and sex risk; 3) cravings for drug use; 4) psychological status (depression, anxiety, symptoms of psychiatric disorder); 5) confidence in and satisfaction with MET, BUP/NX or XR-NTX treatment; 6) behavioral management strategies to be implemented until the next counseling session. Counseling sessions are delivered by trained and experienced staff guided by a counseling manual that has been developed for the NIDA-funded study at Go Vap clinic entitled “A Pilot Implementation Project of Methadone and Suboxone® for Injecting Drug Users in Ho Chi Minh City, Vietnam.” Counselors have worked in the current R01 study at Go Vap clinic and are trained and experienced in counseling heroin users.

3.1.3 Follow Up Phase

All participants will receive directly observed daily medication methadone, buprenorphine or monthly XR-naltrexone injection and cART. All participants will also receive weekly structured manual-based counseling sessions for the first 3 months and monthly counseling sessions thereafter. Each session will last about 45 minutes. Participants will be followed for 48 weeks.

3.1.4 Allocation to Interventional Group

The randomization schedule will be prepared using a randomize block strategy to ensure balance throughout the trial randomization will be performed on demand in real time using a dedicated CDETweb toolbox application, according to the schedule provided by the statistical team.

3.2 Study Endpoints

3.2.1 Primary Study Endpoint

The primary study endpoints will compare plasma sCD14 concentration in HIV-positive opiate use disorder individuals who receive cART and methadone, buprenorphine/naloxone or XR-naltrexone over 48 months

- Plasma assessments (baseline, weeks 4, 8, 12, 24, 36, and 48)
  - sCD14

3.2.2 Secondary Study Endpoints

- To evaluate the change in markers of cellular immune activation and senescence, systemic inflammation and bacterial translocation.
  - Immune activation and senescence. These variables will be assessed by flow cytometry, ELISA or rtPCR on cryopreserved PBMC and plasma, at the indicated time points.
    - Flow cytometry (baseline, weeks 4, 8, 12, 24, 36 and 48)
      - CD38, HLA-DR and PD1 expression in C8+ T cells
      - Activation signature in monocytes
      - CD169 expression in monocytes
    - Plasma assessments (baseline, weeks 4, 8, 12, 24, 36, and 48)
      - sCD163
    - rtPCR-based assessments (baseline, weeks 12, 24, 36, and 48)
      - Type-I IFN signature in PBMC
  - Inflammation will be assessed using Luminex plexes on cryopreserved plasma at baseline, weeks 4, 8, 12, 24 and 48.
    - Acute phase reactants: Plasma hr-CRP and d-dimer
    - Cytokines and receptors: sTNFR-1, IL-6, IL-10, TGF-beta
  - Dysbiosis and bacterial translocation will be evaluated at baseline and at week 48 in plasma (ELISA) and stool samples (rtPCR).
    - Plasma based assessments: sCD14, LBP, LPS, endo-CAB, Intestinal fatty acid-binding protein (I-FABP), Zonulin-1
  - Stool-based assessments: s16 rDNA, bacterial Butyryl-CoA-CoA

- To evaluate the effectiveness of the treatment administered towards a) reducing/suppressing opiate injection behavior and promoting adherence to the treatment schedule and b) suppressing HIV replication

  Clinical and virological outcomes that will allow us to assess the effectiveness of the treatment administered towards a) reducing/suppressing opiate injection behavior and promoting adherence to the treatment schedule and b) suppressing HIV replication.

  The clinical outcomes will be:
  - CD4 count (clinical indicator of successful viral suppression and immune reconstitution, assessed at baseline, weeks 4, 8, 12, 24, 36, 48)
  - Retention in care (% of completed medication visits)
  - cART adherence (% of pharmacy pickups)
  - Continued drug use (% of positive monthly urine opiate tests or positive self-report)
  - Acceptability of MOUD (qualitative assessment based on satisfaction questionnaire)
  - Virological outcomes (assessed in PBMC or plasma)
  - Viral load suppression (% of randomized individuals achieving VL <50 copies/ml; (% of exposed individuals achieving VL <50 copies/ml)
  - Cell-associated DNA, RNA species (poly-A, multi-sliced Tat-Rev), replicative index (linear measures indicating residual viral replication)

4 Study Population and Duration of Participation

4.1 Inclusion and Exclusion Criteria

Inclusion criteria	Exclusion criteria
Age: male or female subjects 18- 65 years of age	Current cognitive impairment, schizophrenia, paranoid disorder, bipolar disorder not compatible with study procedure (assessed by the medical director of the study)
Meet DSM-5 criteria for moderate to severe opiate use disorder (as determined by DSM-5 checklist)	Known neurological, cardiovascular, renal, or other significant medical disorder that is likely to impair or make the individual's participation hazardous
Opiate use with a positive urine drug screen for heroin or other opiates (other than methadone, buprenorphine, buprenorphine/naloxone) at screening visit	Active Tuberculosis or other symptomatic infectious disease
Documented HIV-1 infection with CD4 less than 350 cells/ µL and VL more than 10,000 copies/mL	AIDS-defining illness
cART-naïve or or on cART no longer than 3 months if already started	Current[1] cancer or other malignancies
Willingness to receive cART or on cART no longer than 3 months if already started	Advanced liver disease (FibroScan® METAVIR score F3-F4, liver elasticity more than10kPa)
Willingness to be randomized to either daily methadone, buprenorphine/naloxone or monthly injection of extended-release naltrexone treatment	Use of immunomodulators
Ability to understand and complete study procedures	Meet DSM-5 criteria for any other substance use disorder (except nicotine)
Provision of adequate locator information that lists all contact information a participant agrees that the research staff may use to reach him/her	Engagement in opiate medication treatment at baseline (methadone, buprenorphine, buprenorphine/naloxone, naltrexone)
All participants must be able to comprehend the purpose of the study and to provide informed consent	Pending legal charges with likely incarceration within next 6 months
Is, in the opinion of the study physician, in stable health as determined by pre-study physical examination, medical history, ECG, and laboratory evaluations and is likely to complete the study.	Currently participating in another clinical trial
Has a total body weight of more than 50 kg (110 pounds) and a body mass index (BMI) of more than 20 at screening.	
Female subjects: 1. Cannot be pregnant 2. Cannot be lactating 3. Must be unable to conceive (i.e., surgically sterilized, sterile, or post-menopausal defined as 1 year without bleeding or spotting) OR must agree to use an acceptable method of birth control (e.g., birth control pills, intrauterine device [IUD], or a double barrier method of birth control (condoms and spermicide together; or diaphragm, condom and spermicide together)	

[1] Current is defined as in the last 5 years.

4.2 Subject Recruitment

The study will take place at the Go Vap HIV clinic in Ho Chi Minh City, Vietnam. The Go Vap clinic opened in April, 2013 and provides outpatient treatment for opiate addiction, HIV treatment (antiretroviral, cART), and tuberculosis treatment. Currently, the clinic is treating 500 patients for opiate use disorder (methadone and BDRC-based counseling) with an 92% retention rate at 12-month and about 1,000 patients receiving antiretroviral (cART). The medical staff is comprised of 5 physicians, 6 nurses, 6 counselors, and 4 pharmacists. Participants in the proposed study will be HIV-infected, opioid dependent drug users (primarily heroin users who inject drugs). Subjects will be recruited as they enter treatment for their HIV infection. Intake staff will be trained on the basic inclusion criteria and will invite potential participants to meet with the research staff who will assess potential eligibility and interest in study participation.
If necessary, we will expand the recruitment to other Ho Chi Minh City clinics with the help of the HIV AIDS Association (HAA).
The participants assigned to XR-NTX arm will undergo supervised 2-week opioid inpatient detoxification at the Thu Duc Addiction Treatment Center (under the supervision of Dr. Do), before receiving their first injection of XR-NTX. After the first injection, follow-up will occur at Go Vap clinic, as outpatient visits, following the same scheduling than MET and BUP/Nx groups.

4.3 Duration of Study Participation

The duration of the study subjects' participation will be 48 weeks.

4.4 Total Number of Subjects and Sites

It is expected that approximately 225 subjects will be enrolled in order to produce 158 evaluable subjects. All subjects will be enrolled at the Go Vap Clinic.

4.5 Vulnerable Populations:

Children, pregnant women, fetuses, neonates, or prisoners are not included in this research study.

5 Study Intervention

5.1 Description

All the participants will receive cART and counseling sessions.
Depending on the arm, they will receive either:

- Methadone hydrochloride oral concentrate available in Vietnam is provided by Mallinckrodt PLC.
- Buprenorphine/naloxone sublingual tablets (Suboxone®) provided by Indivior, Inc.
- Injectable extended-release naltrexone (Vivitrol®) administered intramuscular once a month. provided by Alkermes, Inc.

5.2 Receipt of Medication

Methadone, and ART are already available in Vietnam and dispensed by the Go Vap clinic's pharmacy.
XR-NTX (Vivitrol®) is provided by Alkermes Inc. Alkermes will ship the medication to EMINENT Services Corporation (https://www.emiserv.com). EMINENT is a provider of Pharmaceutical, Biological and Information Technology Services for pharmaceutical and biotech Industry and Research Organizations including Federal Government. They have extensive experience with investigational drug formulation, development, manufacturing, testing, packaging and distribution. EMINIENT will receive Vivitrol® from Alkermes and will be in charge to package and to ship the medication to Ho Chi Minh City, Vietnam. The medication will be shipped to YTECO. YTECO will be in charge to receive the medication, to verify and to assure the quantity and quality of the medication. YTECO will be in charge of the storage of the medication and will prepare the packaging and the shipping to the local clinic upon request by the clinic.
The medications are stored in the clinic in controlled environment and temperature. The medications are locked and are dispensed only by pharmacist as per Vietnamese law. Participants will receive their medication at the clinic from the pharmacist, there are no take-home dose allowed in Vietnam. In the same building, another pharmacy is dedicated to infectious disease treatment (i.e., HIV, Hepatitis C, and tuberculosis). A pharmacist is responsible to deliver the cART medications. The pharmacists at the clinic are responsible upon receipt, to verify the medications, to assure their quantity and quality. The pharmacists are also responsible to log the medications and dose dispensed.

5.3 Storage

All medications will be stored in secured and locked, controlled environment (i.e., temperature, protection from light, sterile conditions).

5.4 Administration and Accountability

MET and BUP/NX will be delivered under supervised dose administration by the clinic's pharmacist following Vietnamese guidelines. The pharmacist will complete a daily log including: subject ID, date administered, amount administered, product remaining, product damaged/destroyed and date of damage/destruction, product returned and date of return.
Monthly injection of XR-NTX will be done at the clinic by the medical supervisor. A log of date administration, amount administered and to which subjects, product remaining, product damaged/destroyed and date of damage/destruction, product returned and date of return will be completed by the clinic's pharmacist.
ART will be delivered monthly by the clinic's pharmacist. The pharmacist will complete a log that gathers: subject ID, date of delivery, product delivered, amount delivered, product remaining, product damaged/destroyed and date of damage/destruction, product returned and date of return.

6 Study Procedures

Study procedures timeline

	Screening	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 13	Wk 14	Wk 15	Wk 16	Wk 17	Wk 18	Wk 19	Wk 20	Wk 21	Wk 22	Wk 23	Wk 24	Wk 25	Wk 26	Wk 27	Wk 28	Wk 29	Wk 30	Wk 31	Wk 32	Wk 33	Wk 34	Wk 35	Wk 36	Wk 37	Wk 38	Wk 39	Wk 40	Wk 41	Wk 42	Wk 43	Wk 44	Wk 45	Wk 46	Wk 47	Wk 48
Inclusion criteria	X																																																
Blood test (screening)	X																																																
Fibroscan®	X																																																
DSM-5 SUD checklist	X																																															
Urine drug screen	X	X		X				X				X				X				X				X				X				X				X				X				X				X	
Pregnancy test (women only)	X																																															
Specific questionnaire - Baseline		X																																														
Methadone induction		X	X	X																																												
Methadone maintenance dose (daily)				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Buprenorphine induction		X																																														
Buprenorphine maintenance dose			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Opiates detoxification (inpatient)		X	X	X																																											
XR-NTX injection				X				X				X				X				X				X				X				X				X				X				X				X	
Counseling sessions		X	X	X	X	X	X	X	X	X	X	X	X	X				X				X				X				X				X				X				X					X
HIV specialist - cART initiation		X	X	X																																											
cART refill (pharmacist log)								X				X				X				X				X				X				X				X				X				X				X	
Methadone adherence (pharmacist log)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Buprenorphine adherence (pharmacist log)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
XR-NTX adherence (pharmacist log)		X				X				X				X				X				X				X				X				X				X				X					X
Medical evaluation		X				X				X				X				X				X				X				X				X				X				X					X
Specific questionnaire - Follow-up						X				X				X				X				X				X				X				X				X				X					X
PHQ-9		X				X				X				X				X				X				X				X				X				X				X					X
Satisfaction with treatment						X				X				X				X				X				X				X				X				X				X					X
Labs (VL, CD4)	X	X				X				X				X												X																						X
Blood sampling		X				X				X				X												X																						X
Stool sampling		X																																														X

6.1 Screening

Clinical staff at the HIV and addiction clinic will identify potential subjects (those with HIV infection and opioid use disorder) and refer them to the research assistant (RA). The RA will describe the study and those who express interest will be referred to the study medical supervisor, or their designate, who will explain the purpose of the study and the procedures in detail, answer questions, and review the consent to participate. After confirming comprehension, the participant will be asked to sign the form.

Informed consent procedure

Study personnel will first introduce potential participants to the study and explain the study procedures. A written informed consent form (ICF) in Vietnamese, the content of which will be approved by the local Ethics Committee according to local regulations, will also be provided to the candidates, who will be allowed time to read it and discuss it with the study personnel. ICFs will clearly state that participation in the research is completely voluntary and that there will be no consequence to the participant for declining participation or absences from study procedures.

Candidates who consent to participate will sign and date the form; signed ICFs will be locked-stored at the Go Vap site according to local regulation and GCP directives.

Screening
A clinic nurse will draw blood (phlebotomy) to measure HIV viral load and CD4 and to confirm eligibility for cART. Another blood sample will be collected for liver function test (measures of AST, ALT and total bilirubin). The measures will be performed at the Pasteur Institute of Ho Chi Minh City.
All the participants will be evaluated at the clinic by the study medical director who will:

- Conduct physical examinations
- A urine pregnancy test will be performed for female subjects
- Review laboratory work
- Document current opiate use and DSM-5 opiate use disorders using the DSM-5 Substance Use Disorder Checklist. This form is a listing of the eleven DSM-5 criteria for substance use disorder. A score of 4-6 is considered moderate severity and from 7-11 is considered severe substance use disorder. An opioid use disorder score of 4 or greater will be required for study eligibility. We will screen for Opiates, Alcohol and benzodiazepine use disorder. Moderate to severe alcohol or benzodiazepine use disorder will be exclusionary.
- Conduct a clinical interview to rule out psychiatric disorders that exclude participation.
- Perform a Fibroscan® for each potential participant, and those with a METAVIR score F3-F4 will be excluded from the study and will be referred to hepatitis C specialist for care.

6.2 Study Intervention Phase

6.2.1 Visit 1 (Baseline visit)

After the enrollment process, including consent form and screening tests for inclusion/exclusion criteria, participants will be randomized 1:1:1 to MET, BUP/NX or XR-NTX. The randomization schedule will be prepared using a random-size block strategy to ensure balance throughout the trial. The randomization will be performed on demand in real time using a dedicated CDETweb toolbox application, according to the schedule provided by the statistical team.
If the participant is assigned to a treatment that they refuse to enter after randomization, the participant will be allowed to switch to another arm upon consultation and approval by provider.
If a participant switches immediately after randomization or after induction (detoxification or dose adjustment, depending on the arm), he/she will be kept on study, and will be treated as part of the switch arm in an “as treated” analysis (since there would be very little or no exposure to the original treatment), but will be failed in ITT analysis (cf. 8.5).

The study medical director will then coordinate the care at the clinic for cART (with HIV specialist) and MOUD. A treatment plan with scheduled visits will be delivered to the participant.

RA will complete baseline assessment that includes:

- Locator Data Collection: A basic philosophy of the research approach to be implemented is that follow-up begins at recruitment and enrollment. Thus, a detailed contact sheet (Locator Form) will be completed for all subjects as part of the baseline assessment. This locator form will be updated at each subsequent assessment visit. Participants in the research will also be asked to call or visit the research office in the clinic whenever they have questions about assessment schedules or have updates to provide regarding changes in contact information. Participants will be asked to provide contact information on themselves and three additional people with whom they are most likely to stay in contact. They will also be asked about other places where they might be able to be contacted in the future. This instrument has already been used by the investigators in Vietnam.
- Study Specific Questionnaire: This questionnaire has been developed and used in Vietnam in a previous R01 study. This questionnaire gathers demographic information (e.g. age, gender, race and ethnic identity, marital status, educational level, employment status, income and income sources) and include items from the Addiction Severity Index (ASI) [37] that gathers information on medical and psychiatric status, drug and alcohol use and history of treatment, legal status, family/social relations.
- Risk Assessment Battery (RAB): The RAB provides a brief self-report measure of drug use, injection-related risk behavior, and sexual risk and uses the preceding six months as the time interval of interest [38, 39]. The RAB has been translated into Vietnamese and has been used in the previous R01.

- Urine drug screen: We will use the CLIA waived® ACON Dip-and-Read 8-panel test for THC, cocaine, opiates, amphetamines, PCP, methamphetamine, benzodiazepines, barbiturates, and buprenorphine. The urine sample will be provided using standard procedures with temperature monitoring to preclude tampering and dilution.
- Patient Health Questionnaire (PHQ-9): Given the important role of depression in accessing and adhering to treatments for substance use disorder and other chronic medical problems, we will also assess severity of depression using the 9-item Patient Health Questionnaire (PHQ-9) [40]. This brief assessment asks participants to indicate the frequency of occurrence of each of the nine DSM-IV diagnostic criteria for depression during the previous two weeks.

6.2.2 Visit 2 – Treatment induction

The induction of all the treatments (dose adjustment for MET, BUP/NX, detoxification for XR-NTX) will occur over the following 2 weeks in conformity with international guidelines and applicable Vietnam policy.
Participants assigned to the XR-NTX arm will be provided a two-week inpatient detoxification prior to the first injection of XR-NTX. Participants assigned to MET and BUP/NX will receive daily observed dose of treatment at the clinic.
cART will be initiated according to current Vietnamese policies. Opiate addiction treatment and ART (both administered in same clinic) will be started within two weeks.
On this visit, blood will be drawn by nurse at the clinic. The blood will be sent to the Pasteur Institute of Ho Chi Minh city for cell isolation, cryopreservation (cells, plasma), and target analyses of markers of cellular immune activation and senescence, systemic inflammation and virological markers (primary and secondary outcomes). A stool sample will be collected and send to the Pasteur Institute of Ho Chi Minh city for cryopreservation and analysis.

6.3 Follow Up Phase of the Study

6.3.1 Monthly visit

On week 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 and 48, the RA will complete the follow-up specific questionnaire, RAB, PHQ-9 and a urine drug screen will be performed.

6.3.2 Visit week 4, 8, 12, 24, 36, 48

On these visits, blood will be drawn by nurse at the clinic. The blood will be sent to the Pasteur Institute of Ho Chi Minh city for cell isolation, cryopreservation (cells, plasma), and target analyses of markers of cellular immune activation and senescence, systemic inflammation and virological markers (primary and secondary outcomes).
At week 48, a stool sample will be collected and send to the Pasteur Institute of Ho Chi Minh city for cryopreservation and analysis.
Details on sample collection and utilization could be found on Addendum 1.

6.3.3 End of Study Visit

Counseling sessions will help participants plan for ongoing treatment after their participation in the research. Motivational interviewing strategies will be used to encourage participants to remain in treatment. Participants assigned to methadone or buprenorphine treatment will have the opportunity to continue receiving their treatment at the Go Vap clinic or to be referred to another clinic of their choice. Participants on XR-naltrexone arm who want to continue naltrexone treatment will be referred to the Thu Duc Addiction Treatment Center for outpatient oral naltrexone treatment in Ho Chi Minh City. If participants want to switch to methadone or buprenorphine treatment, referral will be made either at Go Vap clinic or at a clinic of their choice.

6.4 Subject Withdrawal

Subjects may withdraw from the study at any time without impact to their care. They may also be discontinued from the study at the discretion of the Investigator for lack of adherence to intervention or study procedures or visit schedules. The Investigator may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented whether or

not each subject completes the clinical study. Subjects who withdraw early will have one final visit to follow up regarding adverse events.

6.5 Early Termination Visits

If a subject decided to leave the study early or is asked by the investigator to cease participation in the study. Motivational interviewing strategies will be used to encourage subjects to remain in treatment and appropriate referral will be made.

7 Statistical Plan

7.1 Primary Endpoint

- The primary study endpoints will compare plasma sCD14 concentration in HIV-positive opiate use disorder individuals who receive cART and methadone, buprenorphine/naloxone or XR-naltrexone over 48 months
  - Plasma assessments (baseline, weeks 4, 8, 12, 24, 36, and 48)
    - sCD14

7.2 Secondary Endpoints

- To evaluate the change in markers of cellular immune activation and senescence, systemic inflammation and bacterial translocation.
  - Immune activation and senescence. These variables will be assessed by flow cytometry, ELISA or rtPCR on cryopreserved PBMC and plasma, at the indicated time points.
    - Flow cytometry (baseline, weeks 4, 8, 12, 24, 36 and 48)
      - CD38, HLA-DR and PD1 expression in C8+ T cells
      - Activation signature in monocytes
      - CD169 expression in monocytes
    - Plasma assessments (baseline, weeks 4, 8, 12, 24, 36, and 48)
      - sCD163
    - rtPCR-based assessments (baseline, weeks 12, 24, 36, and 48)
      - Type-I IFN signature in PBMC
  - Inflammation will be assessed using Luminex plexes on cryopreserved plasma at baseline, weeks 4, 8, 12, 24 and 48.
    - Acute phase reactants: Plasma hr-CRP and d-dimer
    - Cytokines and receptors: sTNFR-1, IL-6, IL-10, TGF-beta
  - Dysbiosis and bacterial translocation will be evaluated at baseline and at week 48 in plasma (ELISA) and stool samples (rtPCR).
    - Plasma based assessments: sCD14, LBP, LPS, endo-CAB, Intestinal fatty acid-binding protein (I-FABP), Zonulin-1
    - Stool-based assessments: s16 rDNA, bacterial Butyryl-CoA-CoA

- To evaluate the effectiveness of the treatment administered towards a) reducing/suppressing opiate injection behavior and promoting adherence to the treatment schedule and b) suppressing HIV replication

  Clinical and virological outcomes that will allow us to assess the effectiveness of the treatment administered towards a) reducing/suppressing opiate injection behavior and promoting adherence to the treatment schedule and b) suppressing HIV replication.

  The clinical outcomes will be:
  - CD4 count (clinical indicator of successful viral suppression and immune reconstitution, assessed at baseline, weeks 4, 8, 12, 24, 36, 48)
  - Retention in care (% of completed medication visits)
  - cART adherence (% of pharmacy pickups)
  - Continued drug use (% of positive monthly urine opiate tests or positive self-report)
  - Acceptability of MOUD (qualitative assessment based on satisfaction questionnaire)
  - Virological outcomes (assessed in PBMC or plasma)
  - Viral load suppression (% of randomized individuals achieving VL <50 copies/ml; (% of exposed individuals achieving VL <50 copies/ml)
  - Cell-associated DNA, RNA species (poly-A, multi-sliced Tat-Rev), replicative index (linear measures indicating residual viral replication)

7.3 Sample Size and Power Determination

Assessment of power and sample size was performed using program within PASS 15 for mixed effects models (PASS 15 Power Analysis and Sample Size Software (2017). NCSS, LLC, Kaysville, Utah, USA, ncss.com/software/pass), which uses simulation studies for the estimation of power of detectable differences.

Comparing sCD14 levels between MET, BUP/NX and XR-NTX with a sample size of 50 per group, and 7 measurements over time for each subject, with 80% power and a type 1 error rate of 5%, the detectable effect size for the main effect of group is 0.8, which corresponds to detecting a difference as small as 255989.1. With the same parameters above, the detectable effect size for both the effect of time and interaction effect of group x time is 0.9, which corresponds to detecting a difference as small as 310386.9. The attainable sample size is 150 subjects, 75 per arm, with an expected minimum of 50 per arm retention (100 total) to the 48-week endpoint of the study. We anticipated a larger sample in MET arm (15% dropouts) than BUP/NX and XR-NTX arms (30% dropouts). However, the sample has been calculated to provide sufficient power to show any differences between the two arms. A conservative estimate of the detectable difference was based on 50 subjects per arm and does not consider the partial subject observations. As our analytical approach using Mixed Effect Regression models (MER) allows inclusion of partially observed subjects, our power calculation may be a slight underestimate for the detectable difference of our sample size.

7.4 Statistical Methods

All analyses will be performed under the supervision of the PIs and Dr. Warren Bilker, Professor of Biostatistics at Penn in the Department of Biostatistics, Epidemiology, and Informatics. First, basic descriptor analysis, dropout handling, data missingness, normality, and adjustments for type 1 error analyses will be performed.

The primary outcome of interest is the change in sCD14 over 48 weeks following ART. This marker will be assessed over 7 consecutive time points (baseline, weeks 4, 8, 12, 24, 36, and 48). The primary analyses for this longitudinal data will use the mixed model regression model (MER) with restricted maximum likelihood. The Mixed model analysis accommodates the non-independence of the multiple measures per subject. The models will include a random intercept to allow for different starting points for individuals. A compound symmetry (CS) covariance structure will be specified in the model. The basic models will contain variables for time (of the measurement), group, and a group by time or sex interaction. To further explore these relationships, a semiparametric trajectory clustering methodology will be applied, as implemented in SAS PROC TRAJ. Secondary variables include added immune and viral parameters measured in Aim 1 and Aim 2 such as CD4 count, retention in care (% of participants), ART adherence (% of refills), continued drug use based on urine testing (% of positive tests), and acceptability of MOUD (Questionnaire scores). These will all be assessed at the same time points as the primary outcome unless stated otherwise (baseline, weeks 4, 8, 12, 24, 36, and 48). All analyses will assess equality of the measurements at specific time points across treatment groups (t-tests or Wilcoxon tests for continuous data such as CD4 and chi-square Fisher's exact test for proportions), as well as longitudinal trends in the measurements and how the trends differ between groups (MER with random intercepts) as described above. Binary data will use a mixed model version of longitudinal regression, which is available in STATA using xtmelogit and in SAS using PROC GLIMMIX.

7.4.1 Baseline Data

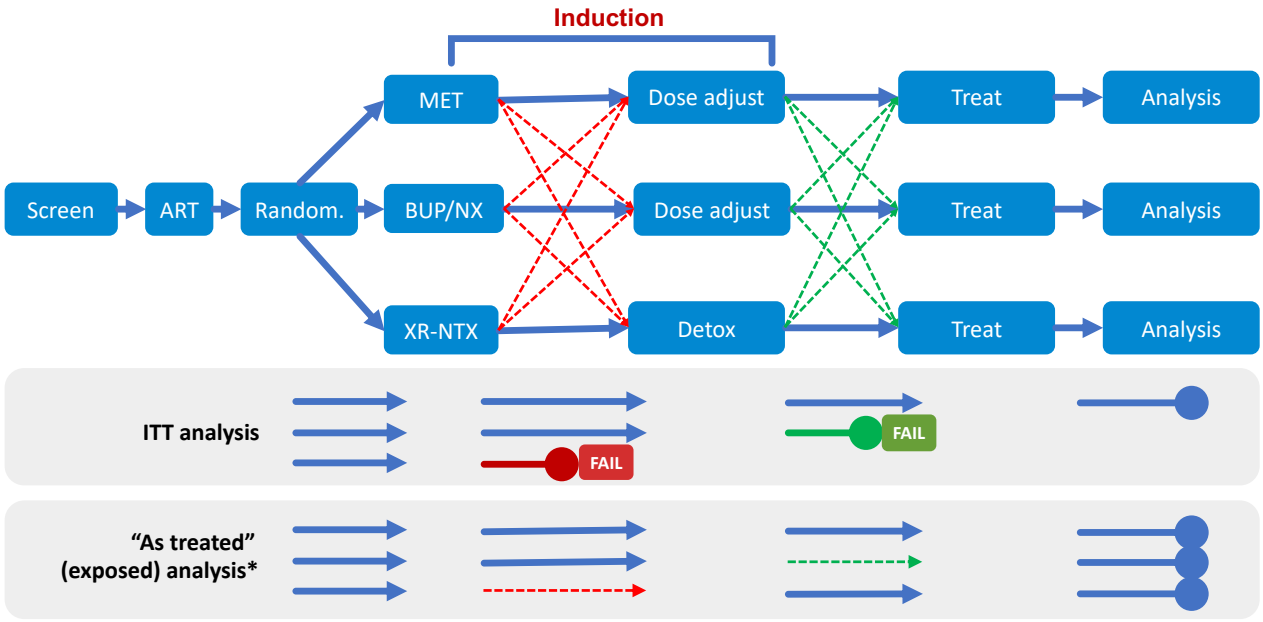
Baseline and demographic characteristics will be summarized by standard descriptive statistics including mean and standard deviation for continuous variables and standard percentages for categorical variables such as gender.

7.4.2 Safety Analysis

All subjects entered into the study and randomized at the baseline visit will have detailed information collected on adverse events for the overall study safety analysis.

7.5 Subject Population(s) for Analysis

Participants that switch immediately after randomization or after induction (detoxification or dose adjustment, depending on the arm) are kept on study, and we treat them as part of the switch arm in an "as treated" analysis (since there would be very little or no exposure to the original treatment), but we fail them in ITT analysis (See schema below). If they want to switch later, they get off study, but we still do the follow-up as needed for safety.



** Arm switching allowed only as an alternative to drop-out, and only within the first 5-8 weeks*

8 Safety and Adverse Events

8.1 Definitions

8.1.1 Adverse Event

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

8.1.2 Serious Adverse Event

Adverse events are classified as serious or non-serious. A serious adverse event is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

All adverse events that do not meet any of the criteria for serious should be regarded as non-serious adverse events.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF).

All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study intervention or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study intervention or study participation will be recorded and reported immediately.

Adverse events that occur at any point in the trial will be identified, managed, and documented.
Medical and scientific judgment should be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject and/or may require intervention to prevent one of the other adverse event outcomes, the important medical event should be reported as serious.

The relationship of each adverse event to the study procedures will be characterized. PIs and medical monitor will classify the AEs/ SAEs as definitely related, probably related, possibly related, unlikely or unrelated to the study.

8.3 Reporting of Adverse Events, Adverse Device Effects and Unanticipated Problems

We will document all adverse events since the subject signs the Informed Consent to the last subject visit. All observed or volunteered adverse events regardless of treatment group or suspected causal relationship to the investigational product(s) will be reported as described in the following sections.

A form will be designed so that there will be a distinction between an “adverse event”, a “serious adverse event” (such as hospitalization, or death), and an important medical event. All adverse events will be documented on this form (along with a description of measures taken in response to the event).

Each report will contain at minimum the following information:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study intervention was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study intervention

Each time an SAE or an important medical event is identified, the project staff member who identified the event will complete an SAE form. The report form will include date, description of the event, duration, severity, measures used to ameliorate effects of the event, and type of referral. The Project Coordinator will be notified immediately. The report form will be reviewed and signed by the Project Coordinator who will be responsible for ensuring that appropriate actions have been taken.

8.3.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator’s assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) will be submitted to the IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

8.3.2 Investigator reporting: notifying the study sponsor

In the event of an SAE or an important medical event, the Principal Investigator (PIs) and the onsite PI will be notified via telephone or email within 8 hours of the event. NIDA Program Officer (Tanya Ramey, MD,

PhD) must be notified within 72 hours since event occurrence. The Principal Investigator will report the event to each IRB within 72 hours of the event occurrence.

8.3.3 Investigator Reporting: Notifying the Penn IRB

Each time an adverse or serious negative event is identified, the project staff member who identified the event will complete an ANEF (Adverse and Negative Events Form). The report form will include date, description of the event, duration, severity, measures used to ameliorate effects of the event, and type of referral. The Project Coordinator will be notified immediately. The report form will be reviewed and signed by the Project Coordinator who will be responsible for ensuring that appropriate actions have been taken.
In the event of an adverse event or a serious negative event, the PIs and the onsite PI will be notified via telephone or email within 8 hours of the event. The PIs will report the event to each IRB within 48 hours. If a serious adverse event occurs, the PI will inform and consult with the Institutional Review Board within 24 hours of that event.

8.4 Unblinding Procedures

Given the exploratory nature of the study, we do not intend to implement blinding across the entire clinical team and participants. However, to minimize bias, both the study Sponsors, all laboratory-based assays, and at least one member of the statistical team will remain blinded. Interim analyses (e.g., for IRBs/Ethics boards) will be structured as closed (unblinded, by-arm reports) and open (aggregated reports). Blinded team members will only have access to open reports.

8.5 Medical Monitoring

The Project Director and the on-site coordinator in HCMC will monitor the regulatory requirements, safety events, and progress of the trial in country. Routine protocol monitoring will ensure that the research protocol specified and EC/IRB approved is being followed without unauthorized deviations. Special emphasis will be placed on ensuring that the consent process is being completed properly and that adverse events are being properly recorded and reported. Periodic assessments of the data quality and timeliness will be undertaken. Participant recruitment and retention will be reviewed. Scientific or therapeutic developments that may have an impact on the safety of the participants or ethics of the trial will be considered. With the assistance of the medical director and the PIs, the team will monitor the confidentiality of the trial data.

Weekly meetings will be held by the study team as well as independent weekly remote meetings between Project Director and on-site staff to monitor the safety and progress of the trial. This will help to ensure standardized application of the protocol and will serve as an ongoing mechanism by which project staff and investigators will communicate to maintain consistently high-quality standards. Concerns identified will be addressed through personnel (re)training. In addition to the weekly monitoring schedule described above, the Project Director will make a formal confirmatory launch site visit (study initiation visit) to supervise staff training and to ensure that the protocol is being followed in all respects. Interview space will be confirmed as adequate, data collection storage capacity will be evaluated, sample route confirmed, and laboratories will be visited to ensure quality control.

8.5.1 Data and Safety Monitoring Plan

A Data and Safety Monitoring Plan describes the process to identify and mitigate potential risks to research subjects.

- The Principal Investigators will monitor the study twice a year or more often if any issue arises
- The Medical monitor will monitor the project on a quarterly basis or more often or more often if any issue arises
- The Project Director will do a monitoring on-site visit twice a year or more often or more often if any issue arises
- The Center for Studies of Addiction Data Safety and Monitoring Board (DSMB) at the University of Pennsylvania will review the procedure, recruitment and retention, AEs and SAEs every 6 months.

8.5.2 Data Safety Monitoring Board

The investigators recognize their responsibility to minimize risk to participants in this investigation and to conduct the study to the highest ethical standards. The project will be reviewed by the University of

Pennsylvania Data Safety and Monitoring Board.

9 Study Administration, Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI

Participation is voluntary, and individuals may withdraw from the study at any time. For those who meet inclusion criteria, detailed informed consent procedures will outline the nature of participation, all risks, and the schedule for data collection and compensation. Potential participants will then be given a detailed description of the research procedures, and all remaining questions will be answered by the research staff prior to completion of consent procedures and initiation of data collection. Subjects will be reminded that they are free to decline or stop participation at any time. All interviews will be conducted on the premises of the Go Vap clinic.

Particular attention will be given to the protection of the confidentiality of any information shared by participants with study personnel. The study procedures are designed to prevent unintentional disclosure of personal information and loss of confidentiality. Staff will be trained on these procedures and on the priority of protection of privacy and confidentiality. Personal Health Information (PHI, as defined by HIPAA) will be collected locally with the purpose of identifying, scheduling and monitoring study participants, as well as for Pharmacy management purposes. Handling of PHI will be limited to the local study team: only partially deidentified limited dataset will be shared with the study Data Management and Statistical teams. If necessary, reidentification of study Participants will be performed by the local study team. Study-related limited datasets will be collected centrally by submitting electronic clinical report forms (eCRFs) available through our proprietary CDETweb Toolbox platform. This web-based platform is developed by Formed, Inc. (Philadelphia, PA) according to industry best practice, and has been audited for HIPAA and 21 cfr part 11 compliance. Our web host (Rackspace, Inc., San Antonio, TX) is HITECH and HITRUST™ CSF certified for HIPAA compliance.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Data Collection and Management

- Data acquisition and transmission

All information will be derived from biological tests, standard clinical procedures, standardized interviews, and self-report questionnaires. All subjects will receive a unique participant number that will be kept separate from any personal information that includes identifying variables such as name, address, relatives, place of work, etc. It is necessary for subject retention that personally identifying material be kept.
All data collection will be conducted at the Go Vap clinic, Ho Chi Minh City, Vietnam under the direct supervision of the site Principal Investigator, who has extensive experience supervising research and implementation and data collection. Protocol monitoring will ensure that the research protocol specified is being followed without unauthorized deviations. Special emphasis will be placed on ensuring that the Consent Process is being completed and documented properly, and that adverse events are being properly recorded and reported. Ongoing assessments of the data quality and timeliness will be undertaken. Participant recruitment and retention will be reviewed formally on a monthly basis via conference call. Scientific or therapeutic developments that may have an impact on the safety of the participants or ethics of the trial will also be considered on an ongoing basis.

Weekly meetings will be held to monitor the progress of the trial. These meetings will involve the Project Director, Site coordinator and other study staff, as well as a representative of the Data management team. This will help to ensure standardized application of the protocol and will serve as an ongoing mechanism by which project staff and investigators will communicate in order to maintain a consistently high quality of study conduct. Concerns identified will be addressed through training and retraining of personnel.

- Data entry methods

Dr. Azzoni (The Wistar Institute, Biomedical Research Support Core) will be responsible for providing and documenting appropriate user access to the study eCRFs and database (CDETweb Toolbox) and preventing data security problems, including unauthorized internal access to data, external access to data, and malicious intent to destroy data and systems. The Wistar BRS Core will ensure that only appropriate and authorized personnel are able to view, access, and modify trial data. Modifications to data will be audited though a continuous audit trail, which will document change, user signature, time and date, and reason for change. The BRS Core will also be responsible for optimizing database performance, reliability, and backup.

External, unauthorized access to data will be prevented through cooperative efforts of the database administrator and network and systems administrators, and by active monitoring of account holders' privileges. Standard measures will be employed through network firewall technologies to prevent unauthorized external access to data repositories. CDEweb Toolbox is regularly audited for HIPAA and CFR 21 Part 11 compliance.

- Electronic Data Management System: CDETweb Toolbox

The CDETweb EDMS is a client-server web application. The architecture allows for multiple, asynchronous users. CDETweb modules and eCRFs are web pages and web forms that allow the user to input data and otherwise interact with the system. The system consists of a number of modules, some of which are standardized across all studies (e.g. Log-in and authority check module, patient registration module, etc.), while others are customized to specifically address individual trial and protocol requirements (e.g. Visit Schedule, Visit CRFs, Randomization module, etc.). Authorized users will sign onto the system from the CDETweb login portal. The authentication module will validate the user's credentials determining the user's access-level by querying a central, BRSC-managed administration database. The BRSC will customize core CDETweb modules in compliance with this Study protocol mandates, e.g. subject registration, visit management, and data capture, as well as any specially requested features (e.g. review pages, special reports etc.).

CDETweb Toolbox addresses the following compliance features:

- Validation. A risk-based validation is planned for selected elements of the tool.
- Human Readable Copies. The system will be able to print readable version of all eCRFs
- Protection and retention of records. The central database is hosted by a commercial host with outstanding reputation and HPAA compliant practices
- Audit trails. Table-based audit trails are implements across the system
- Access control. User must log into the system at the beginning of each session. User names and passwords are regulated
- Authority checks. Role based, as well as granular CRUDAL permissions, are implemented.
- Electronic signatures. Records are electronically signed (exclusively during logged-in sessions) at the time of locking. The signature is stored in the Audit trail, and consist of username and timestamp. To lock a record, the User must acknowledge their intention and responsibilities (i.e. they acknowledge that they are signing the form, and the content correspond to the source to the best of their knowledge) by confirming the entry on an overlay screen displaying this information: thus, intention and meaning of the signatures are implied.
- Device checks. Access to CDETweb Toolbox by means of older, non-HTML-5 compliant browsers is not allowed.
- Training. A manual will be provided to the User containing the basic information required to run the tool. Detailed one-on-one "hands on" training will be provided remotely
- Written procedures. SOPs will be developed in collaboration with the Clinical sites to detail the data collection, entry and retrieval process
- System documentation. Development documentation is maintained by our Web Developers (Formed, Inc.). Validation activities will be documented by the Wistar BRSC.

Electronic Clinical Report Forms (eCRFs) will be developed in close consultation with the clinical study team to accurately reflect protocol data acquisition requirements. After testing and verification of product

specifications, the system will be validated according to the BRS Core SOPs. Site representatives (e.g. Study Team members) will be required members of the Validation team.

Where possible, field-based checks will be implemented to improve data quality by checking input type, range etc. Similarly, eCRF sections will be displayed accordingly to prior entries as necessary. Finally, eCRF field completeness checks will be implemented before the user can lock an eCRF.
Access to locked eCRFs will be administered by the Study Data manager in collaboration with the BRSC. CDETweb™ browser support is currently limited to fully HTML5-compliant browsers.

All participants will be registered in CDETweb® and assigned a unique identifier. This identifier will be recorded in the participant's clinical file, and will serve as the only link between HIPAA-protected information (stored exclusively at the clinical site as described below) and the electronic data, which will not include HIPAA-protected identifiers, with the exception of registration and visit/event dates.

All data will be submitted to the central study database using CDETweb forms; the system will be available on a 24/7 basis for asynchronous data input (anticipated system uptime > 98%). Study coordinators at the clinical core will have separate accounts and will enter data according to the study Protocol and data management plan requirements.

9.3 Records Retention

Questionnaires will be entered on the secured database on an ongoing basis. At the end of the subject participation and after verification of the data entry and QA/ Final QA, the paper copy of the evaluation will be destroyed. Consent forms will be securely stored for 3 years after study completion.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

- A Site Initiation Visit (SIV) will be conducted prior to site activation to confirm preparedness for protocol execution, satisfactory site facilities, clarify the applicable regulations and requirements of the protocol, carefully review the process of implementing the protocol at the site and conduct any necessary training prior to activating the site for enrollment.
- Interim Monitoring Visits (IMVs) will be conducted to confirm participants' rights are being protected; the study is being conducted according to the protocol and applicable regulations, including GCP; confirm accurate reporting of participant safety data and study endpoints.
- For-cause visits (FCVs) are conducted to address any unanticipated issues that arise which require training, remediation or other situations in which the site requires assistance.
- A Close-Out Visit (COV) will be conducted to ensure that all study data and other study documentation is complete and accurate and that all study records have been reconciled.
- The study will be monitored on a semi-annual basis buy the DSMB of the Penn Center for Studies of Addiction.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

11 Ethical Considerations

This study is to be conducted in accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

The Principal Investigators and Co-investigators have certifications of completion for required education on the protection of human research participants. Prior to recruiting human participants into the proposed study, the Project Director, Site/Field Coordinator, and personnel who have responsibility for the design and conduct of the study and/or direct contact with human study participants will complete certification requirements on ethical principles involved in the conduct of human subjects' research by completing the online training module on the NIH website or its equivalent. Certificates of training completion will be kept on file in the project office. The PIs and Project Director will ensure that all project staff are oriented to the procedures during their training period.

11.1 Risks

The medications used in this study are FDA-approved, and commonly used for opiate use disorder treatment globally. MET, BUP/NX, and XR-NTX have a very favorable safety profile.

For participants who continue to use opioids or other medications against medical advice, a risk of overdose exists with all three medications. These risks will be carefully explained to all participants as they enter the study and during weekly counseling sessions. These risks do not derive from the medications themselves, but from the continued use of opioids during treatment and after treatment ends. These risks will be clearly explained at enrollment and reinforced at each visit.

Liver toxicity and possible medication interactions between MOUD and cART medications will be actively monitored through a combination of safety testing and clinical interactions (physical examinations, reported symptom assessment, etc.). When needed referrals to medical treatment or specialists will be made.

The risks attributable to the behavioral assessments are considered minimal. Behavioral assessments may lead to embarrassment or discomfort for some subjects. The majority of this information is clinically relevant and could be considered as standard questions in many clinical settings.

With regard to phlebotomy, risks are minimal and limited to soreness or bruising at the site where the blood is collected. Stool sampling present no additional risk.

11.2 Benefits

No benefits beyond those of the current standard of care in Vietnam (methadone) will be promised to participants. Subjects will receive a medication for opioid use disorder (MOUD) for opiate addiction. The discussions with his/her counselor may help him/her gain a better understanding of drug addiction. The counseling may also help him/her become more successful in reducing or stopping drug use and more successful in HIV treatment. Finally, the counseling sessions may give him/her some ideas on how to improve relationships with family and friends.

All subjects will get appropriate HIV treatment at the Go Vap Clinic with enhanced monitoring of biological markers of progression of the disease.

11.3 Risk Benefit Assessment

The subject will receive MOUD for his/her opiate addiction. The discussions with his/her counselor may help him/her gain a better understanding of drug addiction. The counseling may also help him/her become more successful in reducing or stopping drug use and more successful in HIV treatment. Finally, the counseling sessions may give him/her some ideas on how to improve relationships with family and friends.

All subjects will get appropriate HIV treatment at the Go Vap Clinic with enhanced monitoring of biological markers of progression of the disease. Participation in similar studies has been associated with improvement of health conditions and reductions in risky behavior for many subjects.
In addition to potential individual benefits, the proposed study is expected to yield considerable information on biological and behavioral changes associated with the intervention that may inform the design of future treatment approaches, indirectly benefiting PWIDs.
Overall, the potential benefits are expected to outweigh the minimal risks incurred through participation in this study.

11.4 Informed Consent Process / HIPAA Authorization

Clinical staff at the HIV and addiction clinic will identify potential subjects and refer them to the research assistant (RA). The RA will describe the study and those who express interest will be referred to the study medical supervisor who will explain it in detail, answer questions, review the consent to screen, and have the subject sign it after confirming that he/she understands it. Tests to confirm eligibility for cART will be done on site. For those eligible, informed consent will be obtained in writing by the study medical investigator from all study participants prior to any procedure. Study personnel will first introduce potential participants to the study and explain the study procedures. A written informed consent form (ICF) in Vietnamese, the content of which will be approved by the local Ethics Committee according to local regulations, will also be provided to the candidates, who will be allowed time to read it and discuss it with the study personnel. ICFs will clearly state that participation in the research is completely voluntary and that there will be no consequence to the participant for declining participation or absences from study procedures. Monetary compensation for participation will also be fully described in the ICF. Candidates who consent to participate will sign and date the form; signed ICFs will be locked-stored at the Expertise France office near the Go Vap site according to local regulation and GCP directives.

12 Study Finances

12.1 Funding Source

This study is financed through a grant from the US National Institute of Drug Abuse (R01 DA048728-01). Long-acting naltrexone study drug is donated by Alkermes, buprenorphine/naloxone study drug is donated by Indivior, whereas access to methadone and ART will be purchased from local pharmacies.

12.2 Conflict of Interest

All University of Pennsylvania Investigators will follow the University of Pennsylvania and Wistar investigators the Wistar Institute Policy on Conflicts of Interest Related to Research.

12.3 Subject Stipends or Payments

Participants will receive 120,000 VND (about $5.00) at each monthly assessment; this amount has proven acceptable to both participants and administrative staff in our current research and is not considered coercive.

13 Publication Plan

Generally, the University of Pennsylvania and the Wistar Institute recommend that its researchers share data through communication channels such as speaking engagements and publications, postings on laboratory or institutional webpages, or data archives or enclaves, as appropriate.

It is anticipated that the results of this study will be presented at scientific meetings and/or published in a peer reviewed scientific or medical journal. The investigators involved in the proposed protocol will fully adhere to the timeline stipulated in the NIH policy of dissemination of clinical trial information and publicly disseminate the research observations within 30 months from completion of the study.

14 References

1. Banerjee, S., et al., *Opioid-induced gut microbial disruption and bile dysregulation leads to gut barrier compromise and sustained systemic inflammation.* Mucosal Immunol, 2016. **9**(6): p. 1418-1428.
2. Khansari, M., M. Sohrabi, and F. Zamani, *The Useage of Opioids and their Adverse Effects in Gastrointestinal Practice: A Review.* Middle East J Dig Dis, 2013. **5**(1): p. 5-16.
3. Meng, J., et al., *Morphine induces bacterial translocation in mice by compromising intestinal barrier function in a TLR-dependent manner.* PLoS One, 2013. **8**(1): p. e54040.
4. Peng, J., S. Sarkar, and S.L. Chang, *Opioid receptor expression in human brain and peripheral tissues using absolute quantitative real-time RT-PCR.* Drug Alcohol Depend, 2012. **124**(3): p. 223-8.
5. Saurer, T.B., et al., *Morphine-induced alterations of immune status are blocked by the dopamine D2-like receptor agonist 7-OH-DPAT.* J Neuroimmunol, 2004. **148**(1-2): p. 54-62.

6. Chan, Y.Y., et al., *Inflammatory response in heroin addicts undergoing methadone maintenance treatment.* Psychiatry Res, 2015. **226**(1): p. 230-4.
7. Zajicova, A., H. Wilczek, and V. Holan, *The alterations of immunological reactivity in heroin addicts and their normalization in patients maintained on methadone.* Folia Biol (Praha), 2004. **50**(1): p. 24-8.
8. Kunoe, N., et al., *Injectable and implantable sustained release naltrexone in the treatment of opioid addiction.* Br J Clin Pharmacol, 2014. **77**(2): p. 264-71.
9. Kampman, K. and M. Jarvis, *American Society of Addiction Medicine (ASAM) National Practice Guideline for the Use of Medications in the Treatment of Addiction Involving Opioid Use.* J Addict Med, 2015. **9**(5): p. 358-67.
10. Di Paola, A., et al., *Design and methods of a double blind randomized placebo-controlled trial of extended-release naltrexone for HIV-infected, opioid dependent prisoners and jail detainees who are transitioning to the community.* Contemp Clin Trials, 2014. **39**(2): p. 256-68.
11. Ho, W.Z., et al., *Methylnaltrexone antagonizes opioid-mediated enhancement of HIV infection of human blood mononuclear phagocytes.* J Pharmacol Exp Ther, 2003. **307**(3): p. 1158-62.
12. Springer, S.A., et al., *Extended-Release Naltrexone Improves Viral Suppression Among Incarcerated Persons Living With HIV With Opioid Use Disorders Transitioning to the Community: Results of a Double-Blind, Placebo-Controlled Randomized Trial.* J Acquir Immune Defic Syndr, 2018. **78**(1): p. 43-53.
13. Tetrault, J.M., et al., *Hepatic safety and antiretroviral effectiveness in HIV-infected patients receiving naltrexone.* Alcohol Clin Exp Res, 2012. **36**(2): p. 318-24.
14. Mitchell, M.C., A. Memisoglu, and B.L. Silverman, *Hepatic safety of injectable extended-release naltrexone in patients with chronic hepatitis C and HIV infection.* J Stud Alcohol Drugs, 2012. **73**(6): p. 991-7.
15. UNAIDS. *Country progress Report: HIV and AIDS estimates, Vietnam*. 2018; https://www.unaids.org/en/regionscountries/countries/vietnam].
16. Hoang, T.V., et al., *Impact of a methadone maintenance therapy pilot in Vietnam and its role in a scaled-up response.* Harm Reduct J, 2015. **12**: p. 39.
17. Vietnam Administration of HIV/AIDS Control, *Methadone Maintenance Treatment Vietnam*. 2017.
18. Rangarajan, S., et al., *Risk factors for delayed entrance into care after diagnosis among patients with late-stage HIV disease in southern Vietnam.* PLoS One, 2014. **9**(10): p. e108939.
19. Low, A.J., et al., *Impact of Opioid Substitution Therapy on Antiretroviral Therapy Outcomes: A Systematic Review and Meta-Analysis.* Clin Infect Dis, 2016. **63**(8): p. 1094-1104.
20. Mlunde, L.B., et al., *Association of opioid agonist therapy with the initiation of antiretroviral therapy - a systematic review.* Int J Infect Dis, 2016. **46**: p. 27-33.
21. Sereno, L., et al., *Epidemiology, responses, and way forward: the silent epidemic of viral hepatitis and HIV coinfection in Vietnam.* J Int Assoc Physicians AIDS Care (Chic), 2012. **11**(5): p. 311-20.
22. Dunford, L., et al., *Hepatitis C virus in Vietnam: high prevalence of infection in dialysis and multi-transfused patients involving diverse and novel virus variants.* PLoS One, 2012. **7**(8): p. e41266.
23. Nguyen, C.H., et al., *Prevalence of HBV infection among different HIV-risk groups in Hai Phong, Vietnam.* J Med Virol, 2011. **83**(3): p. 399-404.
24. World Health Organization, *Global tuberculosis report 2014*. 2014, WHO: Geneva, Switzerland.
25. Quan, V.M., et al., *Risks for HIV, HBV, and HCV infections among male injection drug users in northern Vietnam: a case-control study.* AIDS Care, 2009. **21**(1): p. 7-16.
26. Quan, V.M., et al., *Mortality and HIV transmission among male Vietnamese injection drug users.* Addiction, 2011. **106**(3): p. 583-9.
27. Tanuma, J., et al., *Incidence of AIDS-Defining Opportunistic Infections and Mortality during Antiretroviral Therapy in a Cohort of Adult HIV-Infected Individuals in Hanoi, 2007-2014.* PLoS One, 2016. **11**(3): p. e0150781.
28. Nguyen, T., et al., *Change over 12-months of an Integrated Treatment Program for Opiate Addiction and HIV in Ho Chi Minh City, Vietnam: highlighting the role of counseling*, in *NIDA International Conference*. 2017: Montreal, Canada.
29. Hunt, P.W., et al., *Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection.* J Infect Dis, 2014. **210**(8): p. 1228-38.
30. Marchetti, G., et al., *Microbial translocation is associated with sustained failure in CD4+ T-cell reconstitution in HIV-infected patients on long-term highly active antiretroviral therapy.* AIDS, 2008. **22**(15): p. 2035-8.
31. Sandler, N.G., et al., *Plasma levels of soluble CD14 independently predict mortality in HIV infection.* J Infect Dis, 2011. **203**(6): p. 780-90.

32. Sunil, M., et al., *Unchanged Levels of Soluble CD14 and IL-6 Over Time Predict Serious Non-AIDS Events in HIV-1-Infected People.* AIDS Res Hum Retroviruses, 2016. **32**(12): p. 1205-1209.
33. Hunt, P.W., et al., *T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy.* J Infect Dis, 2003. **187**(10): p. 1534-43.
34. Wesson, D.R. and W. Ling, *The Clinical Opiate Withdrawal Scale (COWS).* J Psychoactive Drugs, 2003. **35**(2): p. 253-9.
35. Chawarski, M.C., M. Mazlan, and R.S. Schottenfeld, *Behavioral drug and HIV risk reduction counseling (BDRC) with abstinence-contingent take-home buprenorphine: a pilot randomized clinical trial.* Drug Alcohol Depend, 2008. **94**(1-3): p. 281-4.
36. Chawarski, M.C., W. Zhou, and R.S. Schottenfeld, *Behavioral drug and HIV risk reduction counseling (BDRC) in MMT programs in Wuhan, China: a pilot randomized clinical trial.* Drug Alcohol Depend, 2011. **115**(3): p. 237-9.
37. McLellan, A.T., et al., *The Addiction Severity Index at 25: origins, contributions and transitions.* Am J Addict, 2006. **15**(2): p. 113-24.
38. Metzger, D.S., et al., *Human immunodeficiency virus seroconversion among intravenous drug users in- and out-of-treatment: an 18-month prospective follow-up.* J Acquir Immune Defic Syndr, 1993. **6**(9): p. 1049-56.
39. Navaline, H.A., et al., *Preparations for AIDS vaccine trials. An automated version of the Risk Assessment Battery (RAB): enhancing the assessment of risk behaviors.* AIDS Res Hum Retroviruses, 1994. **10 Suppl 2**: p. S281-3.
40. Spitzer, R.L., K. Kroenke, and J.B. Williams, *Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire.* JAMA, 1999. **282**(18): p. 1737-44.

15 Addendum

Addendum 1

Sample Collection

The collection of biospecimens (a.k.a.: biological samples) from study participants is described in the table "Study procedures timeline" (rows labeled "blood sampling" and "Stool sampling") in section 6 of the Study protocol

A detailed assessment of the specimen collection timeline for each individual participant is provided in Table 3; based on this assessment, we anticipate the following maximum total amount of individual vials (calculated based on 2 ml or $20x10^6$ cells/vial) or boxes (calculated based on a 9x9 layout)

Table 1: Total study sample collection for shipment

Type of Sample	Storage	Per study subject total vials (see Table 3 below)	Total Study vials (225 participants max)	Total shipment boxes (81 samples)
Urine	-70	65	14,625	181
PBMC	Liquid Nitrogen	35	7,875	98
Plasma	-70	35	7,875	98
Swabs	-70	4	900	12
Serum	-70	7	1,575	20
		Grand totals	32,850	409

Sample processing and transfer

All samples will be collected at the point of care and processed at the Pasteur Institute – Ho Chi Minh City (blood separation and cryopreservation, initial Flow cytometry assessment on whole blood, sample aliquots and Swab processing).

Cryopreserved samples will be transferred to the Wistar Institute for analysis. Shipments will be performed using cryogenic Liquid Nitrogen containers ("dry shippers") using a cold chain commercial supplier (e.g. World Courier, Inc.). To maximize efficiency and reduce loss risk, samples will be sent in multiple batches throughout the course of the Study.

Import and export permits will be obtained (or verified if already obtained) from the competent Country Authorities prior to each shipment.

Sample utilization

The samples collected will be necessary to perform the following assessment

Cellular measures of residual immune activation and senescence on ART

An initial analysis of cellular activation (e.g. expression of CD38 and HLA-DR on subsets of memory and naïve CD4+ and CD4+ T cells, as well as markers of activation on CD14+ and CD16+ monocytes) will be performed on 2 ml of fresh whole blood at the Pasteur Institute – Ho Chi Minh City, using 6-color flow cytometry.

In-depth Immunofluorescence profiling

An in-depth secondary analysis will be based on two 16 color composite stains requiring 2 million PBMC each. We will prioritize analysis for pre-ART baseline, week 12, 24 and 48 (4 time points). Details are reported in the budget justification per fluorochromes and antibody clones to include activation changes in T cells and NK cells (PD-1, CD69, CD38, HLA-DR), NK (CD69, Perforin, CD57), monocyte (non-inflammatory: CD14+CD16-, inflammatory: CD14+CD16+) and monocyte/dendritic cell subset (PDL1, CD169, CD40). CD4 T cell HIV correctors will also be measured (CXCR4, CCR5).

Systemic plasma--based measures of residual immune activation/inflammation on ART

The effect of viral suppression (ART) and MAT on systemic activation and inflammation will be assessed measuring specific lymphocytes and acute phase reactants. To assess monocyte activation and microbial translocation, in addition to the PBMC-based stainings mentioned above, we will measure plasma levels of sCD163 and sCD14 using commercial ELISA kits. All time--points will be tested for sCD14 as the primary variable, whereas sCD163 will be tested at pre--ART baseline, week 12, 24, and 48 (4 time points).

Microbial translocation and dysbiosis can be associated with opioid use in PWIDs

Microbial translocation and dysbiosis will be assessed by three methods.
First, plasma levels of LPS: LPS concentration will be determined by the Limulus Amebocyte assay according to the manufacturer's protocol (Cambrex Bioscience, Walkersville, MD) in plasma samples diluted 1/100 with endotoxin-free water.
Second, plasma bacterial 16S rDNA will be measured using droplet digital polymerase chain reaction (ddPCR) using the RainDrop system (RainDance Technologies), which allows significantly higher sample inputs than traditional quantitative PCR. DNA will be extracted from plasma using the DNeasy blood and tissue kit (Qiagen). The RainDrop Source instrument will be used with a microfluidic chip to generate a collection of uniformly sized (5 picoliter) aqueous droplets from each DNA sample mixed with assay reagents in a reaction volume of 50 µl with 25 µl of 2x TaqMan Universal Master Mix II, including UNG (Life Technologies), a final concentration of 450 nM for each primer and 150 nM for the probe, 2 µL 25x Droplet Stabilizer (RainDance Technologies). Droplets will be thermocycled at 50°C for 2 minutes, 95°C for 10 minutes, then 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. Single droplet fluorescence will be measured and data will be analyzed using the RainDrop Analyst software. Intra-assay variability is <5%. We will prioritize analysis for pre--ART baseline, week 12, 24 and 48.
Third, butyryl-CoA transferase levels in the stool (a marker for low representation of butyrate--producing bacterial species associated with immune activation) will be assessed measuring Butyryl--CoA--CoA transferase transcripts by qPCR using SYBR green PCR reagent. These tests will be performed on DNA prepared on site from fresh stool samples (swabs) at baseline and at week 48 to maximize detectable change (2 time points).

Reversal of the activation gene expression signatures in myeloid subsets

Based on our prior characterization of monocyte gene expression changes in associated with HIV-mediated activation and monocyte apoptosis, including genes in the p53 (Rb1, MDM2), Bcl--2 (Mcl2, Bcl--w, Mcl--1, Bax, Bak1, Bik, CycS) and ISG (Mx1, IF6, IFI27, IFITM2) families, we will measure the expression of these genes at baseline and at week 48. This will provide an independent confirmation for the trajectory of changes between week 0 and 48 (primary end--point) and the inferences of retained/decreased innate cell immune activation between arms. Gene expression analysis (RT--PCR) will be performed in CD14+ cells purified from cryopreserved PBMC to greater than 98% purity by negative selection using magnetic beads (Miltenyi Biotech). Total RNA will be isolated and analyzed as described above for HIV measures.

Persistent HIV transcription/replication on ART

In addition to plasma viral load, we will assess the levels of HIV transcriptional activity index (defined as the ratio of cell--associated HIV RNA to cell--associated HIV total DNA). 1--2 million CD4+ T cells will be isolated by negative selection from 20 million PBMCS using EasySep Human CD4+ T cell enrichment kit (Stemcell Technologies). Cellular RNA and DNA will be purified from isolated CD4+ T cells using the AllPrep DNA/RNA kit (Qiagen). Absolute quantification of HIV total DNA, multi--spliced HIV RNA, and

polyadenylated HIV RNA, will be performed, in addition to TERT (telomerase reverse transcriptase; life technologies) as a cell counter, in duplex digital droplet PCR reactions
using the RainDrop system and primers and probe sets as mentioned in the above references. For RNA, cDNA will be generated using SuperScript IV. The RainDrop Source instrument will be used as in Specific Aim 1. Droplet counts will be normalized to TERT counts. We will prioritize analysis for pre--ART, week 12, 24, and 48.
A summary of the specimen utilization is provided in table 2 below: of the anticipated 100 x 10^6 PBMC, we will use 60 x 106 in the tests below, and store 40 x 10^6 cells in case tests need to be repeated, or to compensate for insufficient cell recovery/loss of viability after thawing.

Table 2: Sample utilization

Specific Aim	Description	Tests	Method	Specimen	Amount	Comments
1	Cellular measures of residual immune activation and senescence on ART	Immunostaining w/ 6-color mAb panels	6-color Flow cytometry	fresh blood	1 ml	Pasteur Institute HCMC
	In-depth Immunofluorescence profiling	Immunostaining w/ additional mAb panels	16-color Flow cytometry	PBMC	20 M	n/a
	Systemic plasma--based measures of residual immune activation/inflammation on ART.	Multiple analyte detection	Luminex/ELISA/Metabolomics	plasma	1 ml	n/a
	Microbial translocation and dysbiosis can be associated with opioid use in PWIDs	LPS	Biotest (limulus)	plasma	1 ml	n/a
		Bacterial rDNA	ddPCR	plasma	1 ml	n/a
		Butyryl CoA-CoA transferase	PCR	rectal swabs	1 ml	University of CO
	Reversal of the activation gene expression signatures in myeloid subsets	Rb1, MDM2, Mcl2, Bcl-w, Mcl-1, Bax, Bak1, Bik, CycS, Mx1, IF6, IFI27 and IFITM2	RT-PCR	PBMC	20 M	n/a
2	Persistent HIV transcription/replication on ART	HIV DNA and RNA species on sorted PBMC subsets	ddPCR	PBMC	20M	n/a

Table 3: Biospecimen collection for each individual participant

Sampling	Screen	wk0	wk1	wk2	wk3	wk4	wk5	wk6	wk7	wk8	wk9	wk10	wk11	wk12	wk13	wk14	wk15	wk16	wk17	wk18	wk19	wk20	wk21	wk22	wk23	wk24	wk25	wk26	wk27	wk28	wk29	wk30	wk31	wk32	wk33	wk34	wk35	wk36	wk37	wk38	wk39	wk40	wk41	wk42	wk43	wk44	wk45	wk46	wk47	wk48	per subject total
Urine	1	1		1				1				1				1				1				1				1				1				1				1				1				1				1			13
Blood Science		1				1				1				1												1												1												1	7
Swabs		1																																																	1	2
serum																																																				
Volumes stored	VOL/n																																																		
Urine	10	10	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	130
Blood Science	100	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	100	700
Swabs (n)	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4
serum	2	2	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	14
Vials	n																																																		
Urine (2 ml)	5	5	0	5	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5	0	0	65
PBMC (20M/vial)	5	5	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	5	35
Plasma (2ml/vial)	5	5	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	5	35
Swabs (2ml)	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4
Serum	1	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	7

VALIDATION SAMPLES

CASE REPORT FORM

PATIENT IDENTIFICATION

Patient No.:

In case of emergency, please contact:
Institut Pasteur of Ho Chi Minh-City
00 84 88 20 28 15
00 84 90 38 17 317

BIOLOGICAL SAMPLES

1. Date of the sampling for investigation |__|__| / |__|__| / |__|__|
   D D M M Y Y
2. blood sample taken ☐0 No ☐1 Yes
   2.1. Laboratory validation of the blood sample ☐0 No ☐1 Yes
3. If blood sample is not validated, please comment (IN CAPITAL LETTERS)

4. Urine sample taken ☐0 No ☐1 Yes
   4.1. Laboratory validation of the urine sample ☐0 No ☐1 Yes
5. If the urine sample is not validated, please comment (IN CAPITAL LETTERS)

6. Rectal swap sample taken ☐0 No ☐1 Yes
   6.1. Laboratory validation of the stool sample ☐0 No ☐1 Yes
7. If the rectal sample is not validated, please comment (IN CAPITAL LETTERS)

STUDY TERMINATION

I have reviewed the data contained in this case report form and attest to the accuracy and completion thereof.

8. Date |__|__| / |__|__| / |__|__|
D D M M Y Y

Signature of the principle investigator

Declaration for Exportation of Biological Materials

Material Description / Amount			
Shipment Method	Shipment Date	Does recipient require an import permit? Yes / No	Permit No.
Source Name / Address		Recipient Name / Address	
Applicant Name		Applicant E-Mail	
Applicant Signature / Date			