Clinical Research Protocol

EFFECT OF METHAMPHETAMINE ON RESIDUAL LATENT HIV DISEASE (EMRLHD) STUDY

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Date

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Short-term effects of methamphetamine exposure on residual viral transcription during treated HIV disease

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study participants enrolled under my supervision and providing complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: EMRLHD.03

Protocol Title: Short-term effects of methamphetamine exposure on residual viral transcription during treated HIV disease

Protocol Date: 06/20/2021

Investigator Signature	*Date*

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LIST OF ABBREVIATIONS

AE	Adverse event
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral therapy
CA-US RNA	Cell-associated HIV-1 RNA
CBC	Complete blood count
CFR	Code of Federal Regulations
CMP	comprehensive metabolic panel
CRF	Case report form
DMC	Data Monitoring Committee
ECG	Electrocardiogram
FDA	Food and Drug Administration
GCRC	General Clinical Research Center
GMP	Good Manufacturing Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human Immunodeficiency Virus
ICF	Informed consent form
IEC	Independent Ethics Committee
IRB	Institutional Review Board
LFT	Liver function test
MA	Methamphetamine
NOS	Not otherwise specified
PBMC	Peripheral blood mononuclear cells
PI	Principal Investigator
PID	Participant identification
SAE	Serious adverse experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedures
TSH	Thyroid stimulating hormone
UCSF	University of California, San Francisco
ZSFG	Zuckerberg San Francisco General Hospital

PROTOCOL SYNOPSIS

TITLE	Short-term effects of methamphetamine exposure on residual viral transcription during treated HIV disease
SPONSOR	Investigator Initiated Study (Dr. Sulggi Lee)
FUNDING ORGANIZATION	NIH/NIDA
NUMBER OF SITES	1
RATIONALE	Methamphetamine (MA) use is highly prevalent among HIV+ individuals and is likely to pose challenges for achieving an HIV cure in these individuals, even with effective antiretroviral therapy (ART) suppression. HIV+ ART-suppressed individuals with a history of MA use will be studied to determine whether MA increases virus production and systemic inflammation, and to identify the genes and immunologic pathways that drive MA-induced effects. Data obtained during the course of this study should contribute important and novel genetic and immunologic data to the HIV cure and psychostimulant addiction research agenda.
STUDY DESIGN	This is a phase IV open label double-blind randomized crossover study
PRIMARY OBJECTIVE	1. Determine whether short-term MA exposure increases residual viral production, markers of inflammation (plasma cytokine levels), and cell surface immune marker expression (flow cytometry) in blood.
SECONDARY OBJECTIVES	1. Identify differentially expressed genes and proteins in blood, before and after short-term MA administration. 2. Determine whether trace amine receptor 1 (TAAR1) signaling mediates observed effects of MA administration.
NUMBER OF PARTICIPANTS	N=10
PARTICIPANT SELECTION CRITERIA	Inclusion Criteria: 1. Willing and able to provide written informed consent 2. Male or female, age ≥ 18 years and ≤ 65 years 3. Laboratory confirmed documentation of HIV-1 infection. 4. Continuous therapy with a DHHS recommended/alternative combination ART for least 12 months (at least 3 agents) at study entry with no regimen changes in the preceding 12 weeks 5. Maintenance of undetectable plasma HIV-1 RNA below the limit of quantification for at least 12 months. Episodes of

	single HIV plasma RNA 50-500 copies/ml will not exclude participation if subsequent HIV plasma RNA is below the limit of assay detection. 6. No plans to modify ART during the study period (146 days, or approximately 5 months) 7. Participant and your partner(s) are willing to use two forms of contraception throughout the study period as well as up to 60 days after the last day of study completion 8. Ability and availability to participate in the full 146 days of the study (approximately 5 month) and maintain the inclusion/exclusion criteria 9. No current or prior history of methamphetamine (MA) use disorder by DSM-5 diagnostic criteria. Participants may have a prior history of taking prescription medications containing amphetamines-type stimulants such as Adderall® or Dexedrine® or Ritalin for the treatment of conditions such as attention deficit hyperactivity disorder as long as the participant has not taken these medications in the last 12 months or plans to take these medications during the entire study period. <u>Exclusion Criteria</u>: 1. History of methamphetamine ("meth") use disorder by DSM-5 diagnostic criteria using the 11-symptom checklist. 2. Evidence of MA use other than due to the administered oral methamphetamine study drug, based on urine, hair, or serum MA measurements collected at baseline and follow-up study visits. 3. Current use of prescription medications containing amphetamine-type stimulants (e.g., Adderall®, Dexedrine®, Ritalin, etc.) within the past 1 year. 4. Sensitivity or allergy to amphetamine-type stimulants 5. Current use of any other "psychoactive" drug within the past 1 month. These include cocaine, ecstasy, LSD, mushrooms, or other recreational drugs – but cannabis, nicotine or caffeine use is ok. 6. Use of illicit opioids (heroin, fentanyl) – but ok if use of prescription opioid agonists such as methadone, hydrocodone (Norco®), buprenorphine/naloxone (Suboxone®), oxycodone (Oxycontin®), hydromorphone (Dilaudid®) within the last 3 months by self-report and/or urine qualitative screening.

	7. Current moderate to severe use of alcohol use disorder (DSM-5 criteria) as this might put patient at risk of withdrawal during the study. 8. Recent use within the last month of the following medications given potential interactions with oral methamphetamine: acebrophyline, iobenguane, isocarboxazid, methylene blue, moclobemide, phenylzine, procarbazine, rasagiline, safinamide, selegiline, tranylcypromine, asunaprevir, buproprion, topical cocaine, fluoxetine, iohexol, linezolid, paroxetine, potassium citrate, quinidine, sodium bicarbonate, sodium citrate, sodium lacate, tipranavir, and tromethamine. 9. Recent hospitalization in the last 90 days. 10. Recent infection in the last 90 days requiring prolonged (e.g., >3 weeks) systemic intravenous antibiotics. 11. Known anemia (HIV+ males Hct< 34; females Hct< 32) or contraindication to donating blood. 12. Screening hemoglobin below 12.5 g/dL 13. Poorly controlled hypertension or systolic blood pressure > 140 on repeat measurement in the last 3 months, on more than one occasion. 14. Significant myocardial disease (e.g., current myocarditis or reduced left ventricular ejection fraction below the lower limit of normal) or diagnosed coronary artery disease. 15. History of psychotic symptoms (e.g., hallucinations, delusional thinking) in the prior 3 months. 16. History of seizures, abnormal electroencephalogram or brain damage with significant persisting neurological deficit in the past 3 months or currently on anti-seizure medication. 17. Significant respiratory disease requiring oxygen. 18. Participants of reproductive potential or breastfeeding. Women of childbearing potential must have a negative serum pregnancy test at screening. All participants of childbearing potential must agree to use a double-barrier method of contraception throughout the study period and up to 90 days after the last dose of MA. 19. Exposure to any immunomodulatory drug (including maraviroc) in the 12 weeks prior to study. 20. Prior or current use of experimental agents used with the intent to perturb the HIV-1 viral reservoir in the 12 weeks prior to study. 21. Pregnancy. A serum pregnant test will be performed. If this test is positive, the participant will not be allowed to enter the

	study singe body changes occurring during pregnancy will alter the study results. 22. Recent vaccination within the last 2 weeks prior to study baseline visit. Routine or standard of care vaccinations (such as SARS-CoV-2, influenza, pneumococcal, and meningococcal vaccinations) are allowed but must be administered greater than 14 days prior to baseline study visit. For SARS-CoV-2 vaccination, the last dose must be administered at least 14 days prior to baseline study visit.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Oral methamphetamine hydrochloride (10 mg, then 15 mg) will be administered in a double-blind fashion and prepared by over-encapsulating commercially available drug with cornstarch.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	Placebo capsules will be administered in a double-blind fashion and prepared by over-encapsulating cornstarch.
DURATION OF PARTICIPANT PARTICIPATION AND DURATION OF STUDY	Participants will be randomized to either of two treatment arms, oral methamphetamine or placebo, using a crossover design with a 31-day washout period in between treatment arms. Participants will be on study for up to 146 days. **Screening** (for each arm): up to 14 days prior to treatment arm. **Treatment** (for each arm): 1 day (participants to be admitted to outpatient research unit for a total of 5 hours on the treatment day) **Follow-up** (for each arm): 31 days after the initial dose for that treatment arm. The total duration of the study is expected to be 146 days (approximately 5 months).

CONCOMMITANT MEDICATIONS	Prohibited: Strict criteria for potential medications that could interact with the study drug will be used, and the following medications are not allowed during the study because of potential interactions with the study medication: acebrophyline, iobenguane, isocarboxazid, methylene blue, moclobemide, phenylzine, procarbazine, rasagiline, safinamide, selegiline, tranylcypromine, asunaprevir, buproprion, topical cocaine, fluoxetine, iohexol, linezolid, paroxetine, potassium citrate, quinidine, sodium bicarbonate, sodium citrate, sodium lacate, tipranavir, and tromethamine. This is because the study medication may alter metabolism of the drugs. Allowed: Otherwise, participants may use their usual medications, as long as they are medically necessary and can be monitored safely during the study period.
EFFICACY EVALUATIONS	• HIV virus replication • Immune activation (cell surface immune marker expression by flow cytometry) • Inflammation (plasma cytokine levels) • Gene expression changes (RNA-seq) • Trace amine receptor 1 (TAAR1) signaling
PRIMARY ENDPOINT	• HIV virus replication
SECONDARY ENDPOINTS	• Immunologic effect on inflammation (immune marker expression, plasma cytokine expression). • Gene expression changes • Trace amine receptor 1 (TAAR1) metabolite production
OTHER EVALUATIONS	
SAFETY EVALUATIONS	Incidence of adverse events
PLANNED INTERIM ANALYSES	During the study, safety will be monitored by a Safety Monitoring Committee (SMC), which will be led by an HIV specialist. The SMC will review all study procedures before the initiation of the study. They will have the full and final authority to stop the study for any safety concern at any point. The decision to proceed with further drug administration will be taken by the Principal Investigator, taking into consideration clinical and laboratory adverse events. The decision to proceed with the next dosing level will be taken by the SMC. Specifically, dosing will be stopped and the SMC will be notified if one Grade 4 or two Grade 3 drug related adverse events or laboratory abnormalities are reported, becomes pregnant or initiates

	breastfeeding, or is required to initiate one of the prohibited concomitant medications listed in section 7.1. If stopping/pausing criteria are not met, the remainder of the dosing cohort will be enrolled/dosed the subsequent week. The study will proceed to the next dosing level provided the stopping/pausing criteria are not met. A UCSF Department of Medicine faculty member who is not otherwise involved in the study and who will not communicate with the study investigators regarding any aspect of the SMC reports will prepare the monitoring reports. The SMC will review accrual, adverse events summaries, CD4+ T-cell counts and HIV RNA levels/suppression over time, off-study rates and completeness of follow-up, by dose cohort.
STATISTICS **Primary Analysis Plan**	Dr. Lee has expertise in epidemiology and biostatistics. Differences in residual viral transcription by MA exposure will be evaluated using multivariate negative biniomial regression, as previously described. Potential covariates will include age, gender, nadir CD4+ T cell count, pre-ART HIV RNA, recent CD4+ T cell count, timing of ART (estimated date of HIV infection to date of ART initiation), duration of ART suppression, and other illicit use (stimulant or opioid exposure detected on qualitative urine measurement). Linear regressions will be used to associate MA concentrations with urinary □-PEA levels. For the RNA sequencing data, within-individual differential gene expression analyses will be performed for each tissue type (blood and lymph node) and for each cell type (Tcm CD4+ T cells or CD14+ monocytes). Deconvolution of cell-specific gene signatures69 will be performed to allow analysis of further cell subsets (e.g., “classical” vs. “non-classical” monocytes). For DNA variant analyses, SNP-based association tests between variants and TAAR1 signaling biomarkers will be performed using PLINK/SEQ, while gene-based association tests will be performed using SKAT-O, which aggregates variants across a region and assesses whether the number of variants with non-zero effect sizes within that region exceeds that of chance expectation. Genes and pathways will be filtered for those associated with changes in viral transcription using several pathway sources - e.g., Ingenuity Pathway Analysis (IPA), KEGG, MsigDB (Broad), and Damian Chaussabel’s immune modules. Functional Annotate Network (FAN) will be built using Genes2FANs and Cytoscape to show the key functional network elements that are downstream of candidate genes. String (string-db.org) will be used to build protein-protein interaction networks from the differentially expressed gene lists. Finally, graph theory analysis will be used to identify the functional motifs of the network.

Rationale for Number of Participants	Assuming a log normal distribution of HIV-1 cell-associated (CA)-US RNA and a standard deviation of 0.6 log10 copies/106 CD4+ T cells (as above for Aim 1125), with 10 participants in the treatment arm, and a null hypothesis of no change in CA-US RNA, we estimate that the study will have approximately 80% power to detect a 0.5-0.6 log10 difference in CA-US RNA before and after treatment peak, at the 0.05 significance level. For the gene expression analyses, assuming a coefficient of variation of 0.32 and approximately 80-90% sequencing depth between 5 to 8 copies per base, respectively, we estimate having greater than 80% power to detect a two-fold change at the 0.05 significance level.

1 BACKGROUND

The majority of HIV-infected cells during effective antiretroviral therapy (ART) persist in lymphoid tissues.[1] An effective HIV cure strategy will likely require a combination of potent latency reversal agents to purge this "HIV reservoir" and immunomodulatory agents to boost the host immune response.[2] An additional challenge will be targeting specific marginalized populations who are most likely to benefit from an HIV cure but possess poorer immune responses as a result of residual viral replication due to suboptimal ART adherence[3] and/or illicit substance use.[4] Worldwide, methamphetamine (MA) is the second most commonly used illicit substance,[5] and in San Francisco, up to 39% of HIV+ individuals report recent MA use.[6] These statistics highlight the need to identify an HIV cure in this population but also the importance of determining whether MA use itself may alter the nature of the reservoir or the host immune response.

Prior data shows that MA induces HIV transcription via activation of protein kinase C (PKC) and NF-□B[7-9] and increases systemic immune activation[10-12] and inflammation.[13,14] Our study will directly test in humans the hypothesis that MA use induces residual HIV transcription at the cellular level during effective ART. Our primary objectives are to characterize the reservoir in this population and use this experimental model to determine *in vivo* the molecular pathways that control transcription of the HIV genome.

In support of prior *ex vivo* and animal data demonstrating a link between MA exposure and viral transcription and immune activation, we recently studied 55 HIV+ ART-suppressed participants[15] who use MA and observed that recent MA use was associated with significant changes in the expression of genes associated with HIV latency (*FCGR2A,* encoding CD32a[16] and *PDL1*, encoding the immune exhaustion marker, programmed cell death-1 ligand[17]), as well as genes involved in cell cycle regulation (*FOXO4*[18], *RBM38*[19], *MAPK2*[20]). MA use was also associated with changes in innate immune activation/antigen presentation and inflammation (JAK-STAT signaling) pathways, consistent with prior experimental data demonstrating that MA exposure influences immune activation[10,12,21] and pro-inflammatory cytokine release.[22,23] Although findings from this cross-sectional study support the scientific premise of the proposed study, experimental models are needed to better characterize the mechanisms whereby acute MA exposure alters multiple biological pathways relevant to HIV persistence at the cellular level. As such, the goals of this study are to identify potential interventional targets for future HIV eradication strategies. For example, we will also specifically investigate a clinically promising target for the treatment of psychostimulant addiction,[24-26] trace amine-associated receptor 1 (TAAR1) signaling, and a mechanism by which MA may induce PKC activation and viral transcription.[27,28]

1.1 Overview of Non-Clinical Studies

Prior experimental studies suggest that MA may directly increase HIV transcription and indirectly promote immune activation and inflammation. MA exposure results in increased translocation of NF-κB (a key transcriptional regulator of

the HIV long terminal repeat [LTR] promoter[29]) in primary human microglial cells[7] and leads to dose-dependent increases in HIV reverse transcriptase activity in primary monocyte-derived macrophages.[8] In humanized mice, MA exposure produces a six-fold increase in plasma HIV RNA levels and increased HIV-1 p24 antigen detection in splenocytes.[9] Rhesus macaques administered MA have increased CSF SIV RNA levels, with concomitant increases in the frequency of activated CD14+CD16+ macrophages in the brain.[30] Though no differences in plasma SIV RNA levels were detected in that study, the effect of MA on viral transcription in plasma may have been difficult to detect given that the monkeys were not suppressed on ART (had high plasma viremia overall). MA leads to dysregulated immune activation and inflammation. MA promotes abnormal antigen presentation on phagocytes[12,21] and decreased frequencies of dendritic cells and natural killer (NK) cells in *in vitro* studies.[11,12] MA modifies thymic and splenic cellularity,[31] alters T cell populations,[31] induces apoptotic death,[12,32] and perturbs thymic CD4/CD8 ratios[31,33] in animals. MA leads to the dysregulation of serum cytokine production (e.g., TNF-α, IL-1β, IL-6, IL-2, IL-10, and IL-4) in a mouse model of chronic viral infection.[13] Short-term administration of the methamphetamine-like compound, 3,4-methylenedioxymethamphetamine (MDMA) to healthy human participants reduced immunosuppressive cytokines (transforming growth factor [TGF]-β and IL-10) with a switch to Th2 cytokines (IL-2 and IL-1).[14] Finally, in HIV+ viremic participants, MA use is associated with higher toll-like receptor (TLR)-9 and interferon (IFN)-α protein expression in peripheral blood mononuclear cells (PBMCs), suggesting that MA may also directly activate innate immunity.[10] The proposed Aims 1 and 2 will be the first human *in vivo* studies to longitudinally evaluate the long-term and short-term effects of MA on gene and protein expression changes relevant to inflammatory and innate immune activation pathways.

1.2 Overview of Clinical Studies

A systems biology approach augments the ability to identify host genetic predictors of MA addiction that may be relevant to HIV cure. Prior genetic studies of MA addiction have focused on candidate genes based upon *a priori*-selected genes or pathways, such as genes related to the dopaminergic system like dopamine receptors 2 and 3 (*DRD2*, *DRD3*)[34,35] and dopamine transporter (*DAT*).[36] Two genome-wide association studies (GWAS), which included data from a prior GWAS,[37] found no genome-wide significant single nucleotide polymorphisms (SNPs) associated with MA addiction disorder,[38] though *CDH13* was nominally associated – a gene previously implicated in subjective responses to *d*-amphetamine exposure.[39] Though GWAS genotyping attempts to capture the majority of common SNPs throughout the genome, disease-associated SNPs often localize to "expression quantitative trait loci" (eQTL),[40] regions that are not adequately captured by GWAS genotyping alone. RNA sequencing studies of MA use have been limited and include reported correlates of MA-induced psychosis (upregulation of circadian clock genes: *ELK3* and *SINA3,* and ubiquitin-associated proteolysis: *PIGF* and *UHMK1*)[41] and participant response to topiramate (glutamate and GABA receptor signaling: *GRINA*, *PRKACA*, *PRKCI*, *SNPA23*, *TRAK2*).[42] Most genetic investigations into the etiology of complex traits have primarily focused on only a single genetic datasets at a time. In addition, GWAS data do not provide direct information about the function of these genes.[43] Meta-dimensional analyses integrating DNA, RNA, and protein-level data have the ability to elucidate the molecular mechanisms underlying intricate biological pathways.[44] **For the proposed study, we will use a systems-based approach to prioritize biological pathways that may yield potential novel targets for therapeutic intervention in psychostimulant addiction and HIV persistence.** Despite some understanding now that

host genetics plays an important role in determining MA addiction, the underlying mechanism by which MA induces immune effects and promotes viral production is still incompletely understood. Our data will contribute important DNA, RNA, and protein level data to current public databases that aim to annotate putative regulatory function and tissue-specific regulatory genetic effects.[45-47] The proposed study will use these combined methods to investigate potential direct and indirect effects of MA on viral transcription (possibly mediated by TAAR1) and inflammation in HIV+ ART suppressed individuals.

2 STUDY RATIONALE

Methamphetamine use is associated with poorer clinical outcomes in HIV+ individuals, including decreased adherence to HIV treatment[48] and increased risk of ART resistance.[49,50] In San Francisco, as many as 39% of HIV+ individuals report having used MA in the prior year. The prevalence of MA use in men who have sex with men (MSM) is higher than in the general population, and MA use has been linked to an increased risk of HIV acquisition.[51] Proposed mechanisms by which MA contributes to poorer clinical outcomes include data supporting that MA exposure leads to increased HIV transcription,[7-9] increased T cell activation and exhaustion, and decreased responsiveness to antigenic stimulation.[52] **Yet, little is known about the effects of MA use on viral control during treated HIV disease.** The proposed study will utilize the well-characterized UCSF SCOPE and Options HIV cohorts to perform *in vivo* studies to elucidate how MA exposure influences viral transcription, using cohort and interventional study designs and blood and lymphoid tissue HIV transcription measures. Results from this study may help identify novel targets for HIV eradication and control that are broadly generalizable to HIV+ ART-suppressed individuals, but also specific to those who use MA.

A remaining challenge is targeting vulnerable populations who will most benefit from an HIV cure and yet struggle to receive and remain on treatment. In collaboration with our HIV/AIDS Ward 86 Clinic at the Zuckerberg San Francisco General Hospital (ZSFG) and the San Francisco Department of Public Health, we have now leveraged the city-wide aggressive public health efforts aimed at identifying and immediately treating individuals with newly diagnosed HIV infection, to enroll these individuals into our SCOPE/Options cohorts. However, significant socioeconomic obstacles, such as substance use addiction, make it difficult for these individuals to remain in care and maintain ART suppression. Recently, HIV+ participants with suboptimal ART adherence (i.e., residual viremia below the limit of detection) demonstrate significantly higher levels of plasma biomarkers (e.g., interleukin- 2, -6, and -10; tumor necrosis factor [TNF]-α, and interferon [IFN]-γ).[3] This finding adds new insight into prior data demonstrating that markers of inflammation are significantly higher in HIV+ than in HIV- individuals[53-57] and are associated with higher rates of aging-associated diseases and mortality compared to age-matched HIV- controls.[58,59] Overall, this suggests that inflammation may play a critical role – perhaps mediated by persistent virus itself – even in treated HIV+ individuals, and that in HIV+ individuals who use MA and struggle to maintain optimal ART adherence, aggressively targeting residual viral transcription and inflammation will be critical to achieving a functional cure in these individuals.

2.1 Risk / Benefit Assessment

Side effects associated with taking the study medication

Likely to occur:

- Oral methamphetamine is a stimulant and may cause increased blood pressure, heart rate, and palpitations (noticeably rapid, strong, or irregular heartbeats).

Less likely to occur:

- In some cases, oral methamphetamine can cause some dizziness, headache, insomnia, elevated mood, depressed mood, restlessness, diarrhea, constipation, unpleasant taste, dry mouth, and tremor (hands shaking).

Very unlikely to occur:

- At very high doses (much higher than the doses being given in this study) and if taken for prolonged periods of time (e.g., weeks to months to years), oral methamphetamine can lead to a substance use disorder, exacerbation of preexisting abnormal movements (called "tics"), psychotic symptoms (hearing and seeing things that are not actually there), frequent erections, impotence, and changes in libido.

Side effects associated with taking placebo

Very unlikely to occur:

- In order to make the placebo appear similar to the study medication, the pills will be crushed and placed in a capsule. For the placebo phase, cornstarch will be placed inside the capsule. Therefore, individuals who have an allergy to cornstarch may be at risk of an allergic reaction when taking placebo and should notify the study investigator if they have a known allergy to cornstarch.

HIV viral load and resistance to antiretroviral therapy risks

Very unlikely to occur:

- We will be measuring the amount of viral RNA that increases after the study drug. This does not necessarily mean that there is an increase in the amount of circulating HIV. Nonetheless, since there is a very small chance that there could be an in circulating virus, there would be a theoretical risk of developing resistance to antiretroviral therapy. The likelihood of this is extremely low, given that participants will only be given one day of a single day's maximum pediatric dose of the study medication, and if the study medication induces viral rebound, the predicted increases in HIV in the blood blood is extremely small. For these reasons, individuals can only participate in this study if there are alternate ART regimens available in the rare event that their current ART regimen is compromised as a result of this study. During the study, participants will have their HIV viral load followed closely (at screening, baseline, and on days 2, and 31 of each treatment arm).

Drug-drug interactions

Very unlikely to occur:

- We believe that the likelihood of this is extremely low, given that participants will only be given one day of a low dose of the study medication. Strict

criteria for potential medications that could interact with the study drug will be used, and the following medications are not allowed during the study because of potential interactions with the study medication: acebrophyline, iobenguane, isocarboxazid, methylene blue, moclobemide, phenylzine, procarbazine, rasagiline, safinamide, selegiline, tranylcypromine, asunaprevir, buproprion, topical cocaine, fluoxetine, iohexol, linezolid, paroxetine, potassium citrate, quinidine, sodium bicarbonate, sodium citrate, sodium lacate, tipranavir, and tromethamine. This is because the study medication may alter metabolism of the drugs.

Side effects of taking your blood sample (phlebotomy)

Likely to occur:

- Drawing blood from a vein may cause some discomfort, bleeding or bruising where the needle enters the skin, and rarely, fainting or infection may occur. Up to a total of 795 milliliters (about 53 tablespoons) will be collected during the course of this study, which has been carefully calculated over the 146 days of the study to ensure that blood levels are not below American Red Cross Guidelines.
- Less likely to occur:
- Less common symptoms include lightheadedness, dizziness, fainting and nausea. Symptoms of anemia include tiredness, weakness and dizziness. A CBC with differential will be collected at all visits from screening through day 31 of each treatment arm. If the investigator feels that there is a significant risk for anemia, the amount of blood collected will be reduced. If the participant's hemoglobin falls below 10 g/dl or the hematocrit falls below 27%, there will be a 5 ml (1 teaspoon) blood draw check the hemoglobin and hematocrit. No more blood will drawn until the participant's hemoglobin rises above 10 g/dl or hematocrit rises above 27%

Confidentiality

Participation in research may involve a loss of privacy; however, your records will be handled as confidentially as possible. All study questionnaires and research samples will be coded with a study ID. No personal identifiers such as your name will be used for stored specimens. Dr. Lee and the research staff as well as the UCSF Committee on Human Research will have access to your study records and test results. No individual identities will be used in any reports or publications that may result from this study. California regulations require laboratories to report new cases of tuberculosis, hepatitis B, and hepatitis C infection to the county public health department. The reports include the patient's name, social security number, and other identifying information. Information about these new infections is used to track these diseases statewide and nationwide. Other than this required reporting, your results will be treated confidentially by the study staff. Personally identifying information will not be reported to other departments or agencies.

Risk of genetic testing

Genetic information that results from this study does not have medical or treatment importance at this time. However, there is a risk that information about taking part in a

genetic study may influence insurance companies and/or employers regarding a participant's health. To further safeguard participant privacy, genetic information obtained in this study will not be placed in his/her medical record.

Effect on participating in other studies

Every research study has different requirements for participation, which are known as eligibility criteria. If a participant agrees to take part in this study, the procedures required for this study may make him/her not eligible to participate in other studies for a period of time. How long that period of time is determined by the other study.

Reproductive risks

Methamphetamine is classified as FDA pregnancy risk category C. There are no adequate and well-controlled studies of methamphetamine use in pregnant women. Since there are limited data on pregnancy risk related to methamphetamine given for a single day at the FDA-approved pediatric daily dose, we will take precautions around potential pregnancy during the trial. According to the manufacturer, amphetamines are excreted into breast milk, and women who are taking amphetamines should refrain from nursing. The effect of stimulant medication exposure via breast milk on the neurological development of the infant has not been well studied. Breast milk concentrations in one woman taking 20 mg daily of racemic amphetamine ranged from 55 to 138 ng/mL with milk to plasma ratios of 2.8 to 7.5. For this reason, if a participant is breast-feeding, pregnant or plan to become pregnant, she cannot participate in this clinical trial. Female participants will be asked to take a pregnancy test at the beginning of the clinical trial and before each dose of investigationalproducts in order to determine pregnancy. If a participant is able to become pregnant, twoof the following forms of birth control are required, one of which must be condoms or a diaphragm or cervical cap:

- Condoms (male of female) with or without a spermicidal agent
- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD) with published data showing that expected failure rate is < 1% per year
- Tubal ligation
- Hormone-based contraceptive such as oral birth control pills

Participants are advised that even if they use acceptable forms of birth control during the clinical trial, there is a chance she could become pregnant. If she does become pregnant during the clinical trial she is advised:

- To call/notify the study doctor immediately
- To consult an obstetrician or maternal-fetal specialist
- That she will not be given any additional investigational products
- That she will be followed to determine the pregnancy outcome

If the participant is a male who is sexually active with a woman capable of becoming

pregnant, he must agree to use a medically accepted form of birth control during the

course of this research study. He is advised to inform his partner of the potential harm to an unborn child. The partner needs to know that if she does become pregnant during the study:

- The participant will need to call/notify the study doctor immediately
- She will need to consult an obstetrician or maternal-fetal specialist
- Study staff will ask for her permission to collect information about the pregnancy and the health of the baby. This includes information related to the pregnancy/delivery and obstetrical history.

3 STUDY OBJECTIVES

3.1 Primary Objective

Determine whether short-term MA exposure increases residual viral production, markers of inflammation (plasma cytokine levels), and cell surface immune marker expression (flow cytometry) in blood.

3.2 Secondary Objectives

The second objective is to identify differentially expressed genes and proteins in blood, before and after short-term MA administration.

The third objective is to determine whether trace amine receptor 1 (TAAR1) signaling mediates observed effects of MA administration.

4 STUDY DESIGN

4.1 Study Overview

This is a phase IV open label randomized, double-blinded, placebo-controlled crossover study. A placebo treatment arm will be assigned to participants in a randomized crossover design. HIV+ ART-suppressed individuals with no prior history of MA use disorder will be administered 10mg oral methamphetamine hydrochloride (Desoxyn®), followed by 15 mg methamphetamine hydrochloride two hours later, for a total of 25mg in one 24-hour period (the maximum FDA approved daily dose for the treatment of childhood obesity). Participants will complete the study twice (once on a placebo treatment arm and once with the Desoxyn® treatment arm). Which treatment arm occurs first will be randomly assigned and will include a 31-day washout period between the two phases. The placebo is similar in appearance, consistency, and taste to methamphetamine hydrochloride tablets, USP (Desoxyn®), without any known drug-drug interactions (e.g., changes in pH balance that might affect drug absorption) with HIV antiretroviral therapy.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

There is not a primary efficacy endpoint aim for this phase IV study, though we will be reporting changes in measures of HIV transcription, immune activation, and inflammation.

5.2 Safety Evaluations

Incidence and nature of adverse events

5.3 Other Evaluations (include only if applicable)

PBMCs will be collected for gene expression analyses. Plasma samples will be stored for future PK analyses

6 PARTICIPANT SELECTION

6.1 Study Population

1. HIV-positive adults age ≥ 18 years and ≤ 65 years suppressed on long-term stable antiretroviral therapy, with adequate organ function (per criteria listed below).

6.2 Inclusion Criteria

2. Willing and able to provide written informed consent
3. Male or female, age ≥ 18 years and ≤ 65 years
4. Laboratory confirmed documentation of HIV-1 infection.
5. Continuous therapy with a DHHS recommended/alternative combination ART for least 12 months (at least 3 agents) at study entry with no regimen changes in the preceding 12 weeks
6. Maintenance of undetectable plasma HIV-1 RNA below the limit of quantification for at least 12 months. Episodes of single HIV plasma RNA 50-500 copies/ml will not exclude participation if subsequent HIV plasma RNA is below the limit of assay detection.
7. No plans to modify ART during the study period (146 days, or approximately 5 months)
8. Participant and partner(s) are willing to use two forms of contraception throughout the study period as well as up to 60 days after the last day of study completion.
9. Ability and availability to participate in the full 146 days of the study (approximately 5 month) and maintain the inclusion/exclusion criteria
10. No current or prior history of methamphetamine (MA) use disorder by DSM-5 diagnostic criteria. Participants may have a prior history of taking prescription

medications containing amphetamines-type stimulants such as Adderall® or Dexedrine® or Ritalin for the treatment of conditions such as attention deficit hyperactivity disorder as long as the participant has not taken these medications in the last 12 months or plans to take these medications during the entire study period.

6.3 Exclusion Criteria

11. History of methamphetamine ("meth") use disorder by DSM-5 diagnostic criteria using the 11-symptom checklist.
12. Evidence of MA use other than due to the administered oral methamphetamine study drug, based on urine, hair, or serum MA measurements collected at baseline and follow-up study visits.
13. Current use of prescription medications containing amphetamine-type stimulants (e.g., Adderall®, Dexedrine®, Ritalin, etc.) within the past 1 year.
14. Sensitivity or allergy to amphetamine-type stimulants
15. Current use of any other "psychoactive" drug within the last 1 month. These include cocaine, ecstasy, LSD, mushrooms, or other recreational drugs – but cannabis, nicotine or caffeine use is ok.
16. Use of illicit opioids (heroin, fentanyl) – but ok if use of prescription opioid agonists with known prescribed doses, such as methadone, hydrocodone (Norco®), buprenorphine/naloxone (Suboxone®), oxycodone (Oxycontin®), hydromorphone (Dilaudid®) within the past 3 months by self-report and/or urine qualitative screening.
17. Current moderate to severe use of alcohol use disorder (DSM-5 criteria) as this might put patient at risk of withdrawal during the study.
18. Recent use within the last month of the following medications given potential interactions with oral methamphetamine: acebrophyline, iobenguane, isocarboxazid, methylene blue, moclobemide, phenylzine, procarbazine, rasagiline, safinamide, selegiline, tranylcypromine, asunaprevir, buproprion, topical cocaine, fluoxetine, iohexol, linezolid, paroxetine, potassium citrate, quinidine, sodium bicarbonate, sodium citrate, sodium lacate, tipranavir, and tromethamine.
19. Recent hospitalization in the last 90 days.
20. Recent infection in the last 90 days requiring prolonged (e.g., >3 weeks) of systemic intravenous antibiotics.
21. Known anemia (HIV+ males Hct< 34; females Hct< 32) or contraindication to donating blood.
22. Poorly controlled hypertension or systolic blood pressure > 140 on repeat measurement in the last 3 months, on more than one occasion.
23. Significant myocardial disease (e.g., current myocarditis or reduced left ventricular ejection fraction below the lower limit of normal) or diagnosed coronary artery disease.
24. History of cardiac arrythmia that needs to be medically treated.

25. History of psychotic symptoms (e.g., hallucinations, delusional thinking) in the prior 3 months.
26. History of seizures, abnormal electroencephalogram or brain damage with significant persisting neurological deficit in the past 3 months or currently on anti-seizure medications
27. Significant respiratory disease requiring oxygen.
28. Exposure to any immunomodulatory drug (including maraviroc) in the 12 weeks prior to study.
29. Prior or current use of experimental agents used with the intent to perturb the HIV-1 viral reservoir in the 12 weeks prior to study.
30. Pregnancy. A serum pregnancy test will be performed. If this test is positive, the participant will not be allowed to enter the study since body changes occurring during pregnancy will alter the study results.
31. Recent vaccination within the last 2 weeks prior to study baseline visit. Routine or standard of care vaccinations (such as SARS-CoV-2, influenza, pneumococcal, and meningococcal vaccinations) are allowed but must be administered greater than 14 days prior to baseline study visit. For SARS-CoV-2 vaccination, the last dose must be administered at least 14 days prior to baseline study visit.

7 CONCURRENT MEDICATIONS

Participants may continue to take their usual medications, as long as they are medically necessary and can be monitored during the study period.

7.1 Allowed Medications and Treatments

Participants may not have exposure to any immunomodulatory drug (including maraviroc) in the 16 weeks prior to study or during study period and may not currently be taking experimental latent HIV reversing agents.

Only concomitant medications that are medically necessary will be continued with appropriate monitoring. In addition, the following medications are prohibited during the study and administration will be considered a protocol violation.

Strict criteria for potential medications that could interact with the study drug will be used, and the following medications are not allowed during the study because of potential interactions with the study medication: acebrophyline, iobenguane, isocarboxazid, methylene blue, moclobemide, phenylzine, procarbazine, rasagiline, safinamide, selegiline, tranylcypromine, asunaprevir, buproprion, topical cocaine, fluoxetine, iohexol, linezolid, paroxetine, potassium citrate, quinidine, sodium bicarbonate, sodium citrate, sodium lacate, tipranavir, and tromethamine. This is because the study medication may alter metabolism of the drugs.

8 STUDY TREATMENTS

8.1 Method of Assigning Participants to Treatment Groups

Participants will be randomized to either oral methamphetamine versus placebo treatment first using a random number generator. Whichever treatment the participant receives first, they will receive the other treatment (placebo or oral methamphetamine) for their second treatment phase staring at Day 77 (See Study Events Table in Other Documents).

8.2 Blinding

The order in which participants complete the two treatment phases (i.e., oral methamphetamine and placebo) will be unknown to both the participants and the PI/study team. Both the oral methamphetamine and placebo will be prepared in identical capsules by the UCSF CTSI investigational pharmacist, out of sight of the study participant, study coordinator, and study site PI and administered to the participant to maintain double blinding. The investigational pharmacist will randomize each participant according to the methods described above. In the event that participant experiences an adverse event that requires the identity of the study drug be revealed, the PI will be able to contact the investigational pharmacist to break the blind. In the event that a participant blind is broken, the study PIs will determine the impact upon the un-blinded participant's continued participation in the study and if a replacement participant is needed on a case-by-case basis.

8.3 Formulation of Test and Control Products

8.3.1 Formulation of Test Product

Oral methamphetamine hydrochloride (10 mg, then 15 mg) will be administered in a double-blind fashion and prepared by over-encapsulating commercially available drug with cornstarch; placebo capsules will contain only cornstarch. Study drug will be labeled and stored at <30° Celsius and protected from light. The half-life of oral MA is 4-5 hours, with 62% of drug eliminated in the urine within the first 24 hours, (1/3 as intact drug and the remainder as metabolites).[60] Individuals with chronic, tolerant MA use are reported to take between 250-500 mg and 1000 mg of MA per episode of use.[21] Since participants included in this study will have no prior or recent MA use, they will be considered to be non-tolerant. Thus, given prior data demonstrating physiologic effects[61-63] at doses similar to what is proposed here (maximum of 25 mg oral per day as recommended for the treatment of attention deficit hyperactivity disorder in children[60]), an initial 10 mg will be administered to assess tolerability, followed by a subsequent 15 mg dose two hours later.

8.3.2 Formulation of Control Product

As above; capsules will contain cornstarchonly.

8.4 Supply of Study Drug at the Site

8.4.1 Dosage/Dosage Regimen

An initial 10 mg of oral methamphetamine study drug will be administered to assess tolerability, followed by a subsequent 15 mg oral dose two hours later. For placebo treatment phase, one placebo capsule will be administered orally on treatment day, followed by a second oral placebo capsule two hours later.

8.4.2 Dispensing

All handling of the study drug and placebo - storage, reconstitution, individual dose preparation, and drug accountability will be done by the study investigators and the investigational pharmacist.

8.4.3 Administration Instructions

Dr. Lee and study team will instruct study staff on how to administer the study drug or placebo with a full 6 oz cup of water. Study staff will then administer the study drug or placebo and observe administration and side effects of participants on the treatment days.

8.5 Supply of Study Drug at the Site

The study drug will be purchased through procurement and delivered to the ZSFG Investigational Pharmacy located in the main Inpatient Pharmacy, Rooms 4H3 + 4H6.

8.5.1 Storage

Study drug and placebo storage will be in a cool, dry place, away from strong light, heat, or moisture. For this study, the study drug and placebo will be stored in a locked cabinet in the ZSFG Investigational Pharmacy located in the main Inpatient Pharmacy, Rooms 4H3 + 4H6. Entrance to the pharmacy is secure by electronic pass key only; limited access to pharmacy personnel and housekeeping. Temperature is controlled by air conditioning and monitored to be between 59-86 F. Temperature is recorded daily by reading an alarmed digital thermometer and recording the reading on a daily log. The CRS investigational pharmacist will dispense study drug and maintain the distribution records.

8.6 Study Drug Accountability

An accurate and current accounting of the dispensing of study drug for each participant will be maintained on an ongoing basis by the study investigators. The number of study drug dispensed will be recorded on the Investigational Drug Accountability Record. The study investigators will verify these documents throughout the course of the study.

8.7 Measures of Treatment Compliance

Study participants will be monitored in the Clinical Research Services Center (CRS) for directly observed therapy on the day(s) when study drug or placebo administered. They will be receiving clinical and laboratory assessments on those days for up to 4 hours post-treatment.

9 STUDY PROCEDURES AND GUIDELINES

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the participant.

A full schedule of events is shown in the Study Events Table.

Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medications taken within 30 days prior to screening and entry and a complete history of ART, HIV-1 related vaccines, and immune-based therapies will be documented at the Screening and Entry visits. At each subsequent visit, all additions or discontinuations of prescription medications should be recorded. Actual or estimated start and stop dates should be recorded.

9.1.2 Demographics

Demographic information (date of birth, sex, race and ethnicity) will be recorded at Screening.

9.1.3 Medical History

At Screening, the first Baseline visit (B1) and Entry (Day 0), the medical history must include all diagnoses within the past 30 days and, regardless of when the diagnosis was made, a complete history of chronic conditions, malignancies, and AIDS-defining conditions. Self-reported or documented nadir CD4+ T cell count should be recorded. Any allergies to any medications or their formulations should also be recorded.

9.1.4 Physical Examination

A complete physical examination which includes vitals will be performed by a study physician at Screening. Targeted physical examination which includes vitals will be performed on Baseline, Study Treatment Day(s), and at Study Days 2, 31, 78, and 107..

9.1.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes at Baseline/Screening, Study Treatment Day(s), and at Study Days 2 and 31.

9.1.6 12-Lead Electrocardiogram

A 12-lead electrocardiogram (ECG) will be obtained on treatment days to evaluate for symptomatic, cardiac conduction abnormality/atrioventricular heart block that would warrant medical treatment, including: AV Block-First degree, AV Block-Second degree Mobitz Type I (Wenckebach), AV Block-Second degree Mobitz Type II, AV Block-Third degree (Complete AV block), Conduction abnormality NOS, Sick Sinus Syndrome, Stokes-Adams Syndrome, Wolff-Parkinson-White Syndrome

9.1.7 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF). Criteria for participant management, dose interruptions, modifications, and discontinuation of treatment will be mandated only for toxicities attributable to study drug.

9.2 Clinical Laboratory Measurements

9.2.1 Basic Laboratory Measures

Blood will be obtained and sent to the Zuckerberg San Francisco General Hospital clinical laboratory for complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count), blood chemistry profile (serum sodium, potassium, chloride, bicarbonate, random glucose, blood urea nitrogen, creatinine), and liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase, total bilirubin, direct bilirubin). At screening, additional laboratory measures will include Hepatitis B surface antigen and Hepatitis C antibody, thyroid panel (thyroid stimulating hormone [TSH]), and plasma β-hCG (females only).

9.3 Research Laboratory Measurements

9.3.1 HIV Clinical Laboratory Assay Measures

Blood will be obtained and sent to San Francisco General Hospital clinical laboratory for real-time determination of CD4+ and CD8+ T cell counts and plasma HIV RNA (viral load).

9.3.2 HIV Virologic Research Assay Measures

Cryopreserved PBMCs will be enriched for CD4+ T cells (StemCell, Vancouver, Canada), and DNA/RNA extracted (AllPrep Universal Kit, Qiagen, Hilden, Germany). CA-US and ms RNA will be quantified by an in-house qPCR TaqMan assay using LTR-specific primers,[64,65] and 2-LTR DNA will be quantified using primers specific for the 2-LTR junctions.[66] Participant specimens will be assayed with up to 800 ng total cellular RNA or DNA in replicate reaction wells and copy number determined by extrapolation against a 7-point standard curve (1 – 10,000 cps) performed in triplicate.

9.3.3 Immunophenotyping

We will measure the host and viral transcriptome pre- and post-dosing by performing RNA sequencing (RNA-seq) on isolated CD4+ T-cells and applying validated bioinformatic pipelines for gene expression analysis. Transcriptomic profiling of longitudinal samples will enable characterization of the effects of oral methamphetamine on host gene expression. In addition, consideration of the gene expression data within the context of the aforementioned virologic measurements will allow us to identify specific host transcriptomic correlates of viral reactivation. We hypothesize that the expression of

particular genes (NFkB pathway components, cell-cycle regulators, cell-intrinsic immune factors) will be associated with the degree of viral reactivation.

9.3.4 Plasma cytokine measurement

Cryopreserved plasma from participants will be used to perform custom Meso Scale Discovery (Meso Scale Diagnostics, Rockland, MD) multiplex human inflammatory assays. Analytes will be assayed using an electrochemiluminescence immunoassay technique per the manufacturer's protocol and analyzed on a Meso Scale Discovery Sector Imager 2400 (MSD, Rockville, MD). TNF-α, IL 6, and IL-10 will be analyzed simultaneously in a 3-plex analysis, while TGF-β1, TGF-β2 and TGF-β3 will be analyzed in a separate 3-plex cytokine analysis. The dynamic range for TNF-α, IL 6, IL-10, TGF-β1, TGF-β2, and TGF-β3 per manufacturer's specifications are (in pg/mL): 0.54-3,700; 0.33-2,000; 0.14-3,700; 9.1-37,000; 2.5-38,900; and 1.4-38,600, respectively.

9.3.5 Serum and breath methamphetamine and amphetamine quantification

Amphetamine and methamphetamine concentrations in serum and breath will be determined using a clinically validated liquid-chromatography tandem mass spectrometry (LC-MS/MS) method. Sample preparation consists of acetonitrile protein precipitation, nitrogen-drying of supernatant, and reconstitution in starting LC conditions. For LC, a Shimadzu Prominence UPLC is used with a C18 column and a gradient of mobile phase A (0.05% formic acid) and B (acetonitrile/methanol). Detection is carried out with a SCIEX 4500 QTRAP® tandem mass spectrometer, with an estimated LLOD of 5 ng/mL.

9.3.6 Urine qualitative toxicology

Urine qualitative toxicology testing will be performed at baseline, screening, treatment day, and post-24 hours and post-day 31 after treatment day using a liquid chromatography high mass resolution tandem mass spectrometry (LC-HRMS) method[67] that allows for the identification of drugs and their metabolites in one assay that has enhanced sensitivity/specificity compared to traditional drug screening immunoassays using LC-MS/MS. Prior to analysis, urine samples will be extracted using matrix dilution method, respectively. The data can be retrospectively analyzed for drugs and metabolites of interest given the untargeted acquisition method, with data collected for all compounds within the mass range of 50-700 daltons. The method has been fully validated for the qualitative detection of 250 drugs and metabolites with lower limits of detection (LLOD) ranging from 0.1 – 50 ng/mL.

9.3.7 Hair ART and MA measurements

Concentrations of ART will be quantified from human hair samples at baseline only. Hair ART and MA assessment will be performed using validated liquid chromatography/ tandem mass spectrometry (LC-MS/MS) methods.[68-70] Measurements from this validated assay allow quantification of protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and integrase strand transferase inhibitors (INSTIs) using 20-30 strands of human hair (~1-3 mg) and tenofovir (TFV) and emtricitabine (FTC) from 50-100 strands of hair (~5-10

mg). TFV measurements include tenofovir disoproxil fumarate (TDF) as well as tenofovir alafenamide (TAF).[71] These methods are now being adapted in the UCSF Hair Lab to analyze MA and its metabolites, applying previously published methods,[71,72] and costs have been included in the budget of this proposal to allow adequate ability for the development and validation of these measures

9.3.8 Trace amine receptor 1 metabolite quantification

Beta-phenethylamine (□-PEA) concentrations in urine will be determined using a clinically validated LC-MS/MS method by Dr. Kara Lynch at the ZSFG Toxicology lab; this will include costs to perform assay development to identify additional trace amines, such as tryptamine and □-tyramine.[73] LC will be performed using a Shimadzu Prominence UPLC with a C18 column. Detection will be carried out with a SCIEX 4500 QTRAP® tandem mass spectrometer.

10 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

10.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the participant, for the occurrence of AEs during each participant visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The participant may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The participant is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

The specific adverse events that will be solicited include cardiovascular outcomes, using the Division of AIDS (DAIDS) Adverse Events Grading Criteria (https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Arrhythmia (by ECG or physical examination) *Specify type, if applicable*	No symptoms AND No intervention indicated	No symptoms AND Non-urgent intervention indicated	Non-life-threatening symptoms AND Non-urgent intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Blood Pressure Abnormalities[1] ***Hypertension*** *(with the lowest reading taken after repeat testing during a visit)* *≥ 18 years of age*	140 to < 160 mmHg systolic OR 90 to < 100 mmHg diastolic	≥ 160 to < 180 mmHg systolic OR ≥ 100 to < 110 mmHg diastolic	≥ 180 mmHg systolic OR ≥ 110 mmHg diastolic	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
< 18 years of age	> 120/80 mmHg	≥ 95th to < 99th percentile + 5 mmHg adjusted for age, height, and gender (systolic and/or diastolic)	≥ 99th percentile + 5 mmHg adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
Hypotension	No symptoms	Symptoms corrected with oral fluid replacement	Symptoms AND IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Cardiac Ischemia or Infarction *Report only one*	NA	NA	New symptoms with ischemia (stable angina) OR New testing consistent with ischemia	Unstable angina OR Acute myocardial infarction
Heart Failure	No symptoms AND Laboratory or cardiac imaging abnormalities	Symptoms with mild to moderate activity or exertion	Symptoms at rest or with minimal activity or exertion (e.g., hypoxemia) OR Intervention indicated (e.g., oxygen)	Life-threatening consequences OR Urgent intervention indicated (e.g., vasoactive medications, ventricular assist device, heart transplant)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Hemorrhage (with significant acute blood loss)	NA	Symptoms AND No transfusion indicated	Symptoms AND Transfusion of ≤ 2 units packed RBCs indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs (for children, packed RBCs > 10 cc/kg) indicated
Prolonged PR Interval or AV Block *Report only one* *> 16 years of age*	PR interval 0.21 to < 0.25 seconds	PR interval ≥ 0.25 seconds OR Type I 2nd degree AV block	Type II 2nd degree AV block OR Ventricular pause ≥ 3.0 seconds	Complete AV block
≤ 16 years of age	1st degree AV block (PR interval > normal for age and rate)	Type I 2nd degree AV block	Type II 2nd degree AV block OR Ventricular pause ≥ 3.0 seconds	Complete AV block
Prolonged QTc Interval[2]	0.45 to 0.47 seconds	> 0.47 to 0.50 seconds	> 0.50 seconds OR ≥ 0.06 seconds above baseline	Life-threatening consequences (e.g., Torsade de pointes, other associated serious ventricular dysrhythmia)
Thrombosis or Embolism *Report only one*	NA	Symptoms AND No intervention indicated	Symptoms AND Intervention indicated	Life-threatening embolic event (e.g., pulmonary embolism, thrombus)

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the participant's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.

Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

10.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the participant or require intervention to prevent one of the outcomes listed.

10.2.1 Serious Adverse Experience Reporting

The study site will document all SAEs that occur (whether or not related to study drug) per UCSF CHR Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB), the site investigator will report SAEs to the IRB.

10.3 Monitoring

During the study, safety will be monitored by a Safety Monitoring Committee (SMC), which will be led by a qualified UCSF faculty member in the Department of Medicine with expertise in HIV clinical research (see Section 13 below). The SMC will review all study procedures before the initiation of the study. They will have the full and final authority to stop the study for any safety concern at any point. The Principal Investigator will be the primary medical monitor responsible for study adverse event monitoring and will report adverse events and unanticipated problems to the UCSF IRB and the SMC. All participants will be followed for possible adverse events and unanticipated problems throughout the study period.

Participants will be monitored in an outpatient clinic during the first 6 hours post-dose on dosing days. At each visit, participants will be assessed for any new symptoms, and a study coordinator will obtain vital signs. The study will require 48-hour reporting of all laboratory values, signs/symptoms, and serious adverse events (SAEs). The study will have intensive Phase I safety monitoring, which will include regular and frequent team review (every week for the first 2 months of the study; then every two weeks thereafter) of all reported events. In addition, for any Grade 3 or 4 events, sites will be instructed to contact the study team by email.

- Grade 1 or 2 Toxicity: Participants who develop a Grade 1 or 2 AE or toxicity may continue study treatment. If a participant chooses to discontinue study treatment, the site should notify the study protocol core team as noted above, and encourage the participant to complete any remaining study visits.

- Grade 3 or 4 Toxicity: Participants experiencing Grade 3 or 4 AEs requiring permanent discontinuation of study treatment should be followed closely for resolution of the AE to Grade ≤ 3 and the protocol core team must be notified. Participants discontinuing study treatment should be encouraged to complete any remaining study visits.

Dr. Sulggi Lee will be contacted directly to report medical concerns or questions regarding safety.

Phone: (415) 735-5127

10.4 Criteria for Discontinuation

Safety will be monitored by the dedicated SMC, led by a qualified Drug and Substance Abuse specialist in the UCSF Department of Medicine (see Section 13 below). The SMC will have full and final authority to stop the study at any point for safety concerns. The decision to proceed with further drug administration will be taken by the Principal Investigator, taking into consideration clinical and laboratory adverse events. The decision to proceed with the next dosing level will be taken by the SMC. Specifically,

dosing will be paused and the SMC will be consulted if one Grade 4 or two Grade 3 drug related adverse events or laboratory abnormalities are reported.

Specifically, safety discontinuation criteria will include:

1. Any serious adverse event assessed as at least possibly related to study treatment:
    a. Any Grade 4 drug-related adverse event or laboratory abnormality
    b. Two Grade 3 drug-related adverse event or laboratory abnormality
2. Pregnancy or breastfeeding
3. Requirement for prohibited concomitant medications (see section 7.1)
4. Clinical reasons believed life threatening by the physician, even if not addressed in the toxicity section of the protocol

Study drug-specific hold criteria after the first methamphetamine dose will be based on established protocols adapted from prior stimulant interventional trials (Walsh et. al. *Drug Alcohol Depend*. 2010 Jan 1;106(1):28-37. doi: 10.1016/j.drugalcdep.2009.07.011):

- SBP ≥ 165 mm Hg or
- DBP ≥ 100 mm Hg or
- HR ≥ 130 BPM

Resume methamphetamine administration only if values are below the above parameters for at least 3 consecutive minutes.

Stop further methamphetamine administration if the following are reached for 4 minutes or more:

- SBP ≥ 180 mm Hg or
- DBP ≥ 120 mm Hg or
- HR ≥ submaximal HR (i.e., 220 - [age x 0.85])

Such a participant will be observed until cardiac parameters return to normal and will be discontinued from the study.

In addition, any significant ECG abnormality (determined by monitoring medical staff) will suspend further dosing.

If stopping/pausing criteria are not met, the remainder of the dosing cohort will be enrolled/dosed the subsequent week. The study will proceed to the next dosing level provided the stopping/pausing criteria are not met. A UCSF Department of Medicine faculty member who is not otherwise involved in the study and who will not communicate with the study investigators regarding any aspect of the SMC reports will prepare the monitoring reports. The SMC will review accrual, adverse events summaries, CD4+ T-cell counts and HIV RNA levels/suppression over time, off-study rates and completeness of follow-up, by dose cohort.

11 DISCONTINUATION AND REPLACEMENT OF PARTICIPANTS

11.1 Early Discontinuation of Study Drug

A participant may be discontinued from study treatment at any time if the participant or the investigator feels that it is not in the participant's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Participant withdrawal of consent
- Participant is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the participant to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up
- Sponsor request for early termination of study
- Positive pregnancy test or breastfeeding (females)

If a participant is withdrawn from treatment due to an adverse event, the participant will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All participants who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for participant withdrawals. The reason for the participant's withdrawal from the study will be specified in the participant's source documents (refer to early termination procedures).

12.3 Withdrawal of Participants from the Study

A participant may be withdrawn from the study at any time if the participant, the investigator, or the Sponsor feels that it is not in the participant's best interest to continue.

12.4 Replacement of Participants

Participants who withdraw from the study treatment before last day of study drug dosing will be replaced by another enrolled participant to maintain total study numbers.

The CRF will document the reason for the withdrawal and date of withdrawal. Date of withdrawal will be documented as the date of last study drug treatment, not the date that the decision to withdraw treatment was made.

Participants will be followed after withdrawal from the study for 3 calendar days after cessation of treatment. All adverse events during that period will be reported.

12 PROTOCOL VIOLATIONS

A protocol violation occurs when the participant or the study investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, participant safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Principal Investigator will determine if a protocol violation will result in withdrawal of a participant.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

13 DATA SAFETY MONITORING

Safety monitoring will include team review of adverse events (including all reported signs/symptoms, laboratory abnormalities, diagnoses, and SAEs) and team assessment as to the possible relationship of adverse events to the study treatment. The review will also include assessment any participants with an unconfirmed CD4+ T cell or HIV RNA measurement indicating a potential safety endpoint. Regular team monitoring will also assess early study discontinuations and visit/sample completeness. In addition, study accrual and baseline characteristics of study participants will be reviewed periodically during accrual. After a dose cohort has completed accrual and sufficient follow-up time is available, the team will evaluate whether to dose-escalate and open the next dose cohort to accrual, as described below.

Safety Monitoring Committee (SMC). We have established an independent Safety Monitoring Committee (SMC) for this study which include well-recognized experts in HIV clinical care and research: Dr. David Tompkins (Associate Professor of Medicine, UCSF, and Director of the Division of Substance Abuse and Addiction Medicine) and Annie Luetkemeyer (Professor of Medicine, UCSF, and expert on HIV management and clinical trials). The committee will be chaired by Dr. Tompkins who has experience in the regulatory aspects of clinical trials and will independently deliberate using study data.

The SMC will meet after completion of each dose cohort to review safety data. The meeting will occur no earlier that two weeks after the last dose. The decision to move to the next dose cohort will be made by the SMC, after consultation with the study investigators.

A study data coordinator will produce administrative reports regularly describing study progress including the following: (1) accrual, (2) demographics, (3) study subject status,

(4) laboratory data, and (5) number and type of serious AEs. Reviews will be communicated to the UCSF Committee on Human Research (CHR), study sponsor, and/or federal agencies, as appropriate. The SMC will have access to treatment assignment. The study will be discontinued if the SMC determines that it is in the best interest of the subjects.

Grade 1 or 2 AEs will not result in any change to the study plans. If there is evidence for a Grade 3 AE that is not caused by the study drug, the study will continue as planned. On the other hand, Grade 3 AEs thought to be possibly caused by the intervention and any Grade 4 AEs will result in a hold on any future enrollments until a decision to proceed or to stop the study is made by the SMC

14 STATISTICAL METHODS AND CONSIDERATIONS

14.1 Data Sets Analyzed

Dr. Lee and her computational data specialist will be performing the data analysis for the study.

14.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be summarized by dose level: race, gender, age, height and weight.

14.3 Analysis of Primary Endpoint

Paired analyses using Wilcoxon signed rank tests will be performed to evaluate whether there is a statistically significant change in markers of immune activation (e.g., CD69, CD38, and/or HLA-DR) on CD4+ and CD8+ T cells, as well as log-transformed levels of plasma cytokines (e.g., IL-6, IL-1b, IFN-g) after study drug compared to baseline (reference will be the average of the three baseline measures). Multivariate analyses will then be performed to evaluate the association between study drug and measures of immune activation at post-dosing, using linear mixed effects models, which account for within-participant correlation of observations and allows for greater stability in the estimate of the outcome measures within individuals. Similarly, for measures of the HIV transcription, linear mixed effects regression to compare CA-US RNA levels before and after study drug will be performed. Negative binomial mixed effects regression (a method to analyze over-dispersed count data) to compare plasma HIV RNA levels before and after study drug administration (reference will be the average of three baseline measures).

14.4 Analysis of Secondary Endpoints

Differences in residual viral transcription by MA exposure will be evaluated using multivariate negative biniomial regression, as previously described.[74] Potential covariates will include age, gender, nadir CD4+ T cell count, pre-ART HIV RNA, recent CD4+ T cell count, timing of ART (estimated date of HIV infection to date of

ART initiation), duration of ART suppression, and other illicit use (stimulant or opioid exposure detected on qualitative urine measurement). Linear regressions will be used to associate MA concentrations with beta-PEA levels. For the RNA sequencing data, within-individual differential gene expression analyses will be performed for each tissue type (blood and lymph node) and for each cell type (Tcm CD4+ T cells or CD14+ monocytes). Genes and pathways will be filtered for those associated with changes in viral transcription using several pathway sources - e.g., Ingenuity Pathway Analysis (IPA),[75] KEGG,[76] MsigDB (Broad),[77] and Damian Chaussabel's immune modules.[78] Functional Annotate Network (FAN) will be built using Genes2FANs[79] and Cytoscape[80] to show the key functional network elements that are downstream of candidate genes. String (string-db.org) will be used to build protein-protein interaction networks from the differentially expressed gene lists. Finally, graph theory analysis[81] will be used to identify the functional motifs of the network.

14.5 Sample Size

Assuming a log normal distribution of HIV-1 cell-associated (CA)-US RNA and a standard deviation of 0.6 $\log_{10}$ copies/10^6 CD4+ T cells (as above for Aim 1125), with 10 participants in the treatment arm, and a null hypothesis of no change in CA-US RNA, we estimate that the study will have approximately 80% power to detect a 0.5-0.6 log10 difference in CA-US RNA before and after treatment peak, at the 0.05 significance level. For the gene expression analyses, assuming a coefficient of variation of 0.32 and approximately 80-90% sequencing depth between 5 to 8 copies per base, respectively, we estimate having greater than 80% power to detect a two-fold change at the 0.05 significance level.

15 DATA COLLECTION, RETENTION AND MONITORING

15.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each participant treated with the study drug.

Case report forms (CRFs) will be provided for each participant. Study personnel will enter data from source documents corresponding to a participant's visit into the protocol-specific paper CRF when the information corresponding to that visit is available. Participants will not be identified by name in the study database or on any study documents to be collected by the study investigators, but will be identified by a four-digit patient identification number (PID). If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Principal Investigator is responsible for all information collected on participants enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Principal Investigator. A copy of the CRF will remain at the study site at the completion of the study.

15.2 Data Management Procedures

All data for our proposed study will be managed by the UCSF Data Coordinating Center, which is housed in the Department of Epidemiology and Biostatistics. This center currently serves 25 multicenter cohort and randomized trials throughout the world

The UCSF Department of Epidemiology and Biostatistics complies with federal, state, University, and campus electronic information security requirements through a combination of physical, technical, procedural, and management controls. At the procedural level, all Coordinating Center (CC) personnel sign a confidentiality agreement and undergo security awareness training for HIPAA and the handling of sensitive data. All employees of the University of California, San Francisco were required to obtain Security Awareness Training and implement appropriate security measures. New employees who use computers must take this training. Remote users of the data also receive training from the Data Management Group prior to gaining access to data systems.

15.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the Investigators and study monitors for resolution. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

15.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

The network at UCSF CC is privately maintained, hardware firewalled and none of the workstations or database servers can be directly addressed from outside the Local Area Network. Website communications are encrypted using an SSL certificate. Network OS is Windows Active Directory. Remote access is via SSL-VPN. A network administrator and server administrators support the network and servers and the Data Systems Services Group (developers/database administrators) support the database and web applications. The support team is paged 24/7 when servers or critical data center equipment experiences issues.

All study data is housed at the UCSF CC in a secure server room. The building is locked outside of normal business hours. All system servers are located in a limited access suite fitted with an Access Control System. Within the locked suite is a locked server room fitted with an additional secure door. Only critical Information Systems staff possesses the access code required to enter the room. All who enter the system server room must sign a server room access log in accordance with UCSF CC IT Security SOPs.

Study database access is controlled via two-factor password security. Development workstation access is controlled via Microsoft logon. Once a workstation is accessible,

access to the study data on the SQL server via any development application requires appropriate logon-specific permission assigned in SQL Security Manager. Communication between study servers and client machines on the UCSF network are encrypted using an SSL certificate. All servers are protected from viruses by McAfee VirusScan. This software automatically checks for virus signature file updates from a McAfee FTP site once an hour, and if necessary directly updates itself. All anti-virus software is monitored and network personnel notified in the event that the software stops functioning on a given server.

All study data are stored on SQL servers managed by the UCSF Coordinating Center. All servers are housed in a secure server room.

Web site access: The study web sites are protected by two hardware-based firewalls to shape incoming and outgoing traffic. Access to the study management web site is restricted to approved personnel only. Approved personnel gain access to the system using a multi-layered authentication scheme. A log of all personnel with level of access is kept and updated regularly. Once a clinic site user accesses the system they are only permitted to view data received from their site, with the exception of official aggregate reports. Users are not permitted to view or alter another clinic's data.

Data transmission from web server to client: The UCSF CC currently utilizes Secure Socket Layer (SSL) protocol which protects all data transmission sent over the Internet between the CC IIS Web Server and every client machine which accesses our study web sites.

System Backup

Back-ups: All department workstations and servers are automatically backed-up every night. Back-up systems are monitored daily.

Failover Site: As part of the nightly database maintenance procedures, all SQL databases are backed up to a "failover" site at our co-location facility. This site has copies of the study databases as well as all associated systems required to carry on a study in the event of a disaster at the primary location.

Off-site Storage: Network back-ups are written to tape and sent to an off-site vendor every two weeks, with tapes being rotated every two months.

Recovery/File Restores: If important files or data from the network are accidentally deleted, the IT staff can locate the items and restore data within an hour in most cases.

15.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each participant must be maintained that includes the signed Informed Consent, HIPAA Authorization and copies of all source documentation related to that participant. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing

of the investigational product or for two years after centers have been notified that the IND has been discontinued.

15.6 Participant Confidentiality

In order to maintain participant confidentiality, only a site number, participant number and participant initials will identify all study participants on CRFs and other documentation submitted to the Sponsor.

16 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

16.1 Protocol Amendments

Any amendment to the protocol will be written by the study investigators. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

16.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB of the participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB unconditional approval statement will be transmitted by the Investigator prior to the shipment of study supplies to the site. This

approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

16.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Participants (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each participant prior to entering the participant into the trial. Information should be given in both oral and written form and participants must be given ample opportunity to inquire about details of the study. A copy of the signed consent form will be given to the participant, and the original will be maintained with the participant's records.

16.4 Publications

The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

16.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of participants.
2. Personally conduct or supervise the study (or investigation).

3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to participants or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/participants.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

REFERENCES

1. Yukl SA, Gianella S, Sinclair E, et al. Differences in HIV burden and immune activation within the gut of HIV-positive patients receiving suppressive antiretroviral therapy. *The Journal of Infectious Diseases* 2010; **202**(10): 1553-61.

2. Deeks SG, Autran B, Berkhout B, et al. Towards an HIV cure: a global scientific strategy. *Nature reviews Immunology* 2012; **12**(8): 607-14.

3. Castillo-Mancilla JR, Brown TT, Erlandson KM, et al. Suboptimal Adherence to Combination Antiretroviral Therapy Is Associated With Higher Levels of Inflammation Despite HIV Suppression. *Clin Infect Dis* 2016; **63**(12): 1661-7.

4. Passaro RC, Pandhare J, Qian HZ, Dash C. The Complex Interaction Between Methamphetamine Abuse and HIV-1 Pathogenesis. *J Neuroimmune Pharmacol* 2015; **10**(3): 477-86.

5. CDC. Increasing morbidity and mortality associated with abuse of methamphetamine - United States, 1991-1994. *MMWR* 1995; **44**: 882-7.

6. Marquez C, Mitchell SJ, Hare CB, John M, Klausner JD. Methamphetamine use, sexual activity, patient-provider communication, and medication adherence among HIV-infected patients in care, San Francisco 2004-2006. *AIDS Care* 2009; **21**(5): 575-82.

7. Wires ES, Alvarez D, Dobrowolski C, et al. Methamphetamine activates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB) and induces human immunodeficiency virus (HIV) transcription in human microglial cells. *J Neurovirol* 2012; **18**(5): 400-10.

8. Liang H, Wang X, Chen H, et al. Methamphetamine enhances HIV infection of macrophages. *Am J Pathol* 2008; **172**(6): 1617-24.

9. Toussi SS, Joseph A, Zheng JH, Dutta M, Santambrogio L, Goldstein H. Short communication: Methamphetamine treatment increases in vitro and in vivo HIV replication. *AIDS Res Hum Retroviruses* 2009; **25**(11): 1117-21.

10. Jiang J, Wang M, Liang B, et al. In vivo effects of methamphetamine on HIV-1 replication: A population-based study. *Drug Alcohol Depend* 2016; **159**: 246-54.

11. Saito M, Yamaguchi T, Kawata T, et al. Effects of methamphetamine on cortisone concentration, NK cell activity and mitogen response of T-lymphocytes in female cynomolgus monkeys. *Exp Anim* 2006; **55**(5): 477-81.

12. Harms R, Morsey B, Boyer CW, Fox HS, Sarvetnick N. Methamphetamine administration targets multiple immune subsets and induces phenotypic alterations suggestive of immunosuppression. *PLoS One* 2012; **7**(12): e49897.

13. Sriram U, Haldar B, Cenna JM, Gofman L, Potula R. Methamphetamine mediates immune dysregulation in a murine model of chronic viral infection. *Front Microbiol* 2015; **6**: 793.

14. Pacifici R, Zuccaro P, Hernandez Lopez C, et al. Acute effects of 3,4-methylenedioxymethamphetamine alone and in combination with ethanol on the immune system in humans. *J Pharmacol Exp Ther* 2001; **296**(1): 207-15.

15. Carrico AW, Flentje A, Kober K, et al. Recent stimulant use and leukocyte gene expression in methamphetamine users with treated HIV infection. *Brain Behav Immun* 2018; **71**: 108-15.

16. Descours B, Petitjean G, Lopez-Zaragoza JL, et al. CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. *Nature* 2017; **543**(7646): 564-7.

17. Banga R, Procopio FA, Noto A, et al. PD-1(+) and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. *Nat Med* 2016; **22**(7): 754-61.

18. Kops GJ, Medema RH, Glassford J, et al. Control of cell cycle exit and entry by protein kinase B-regulated forkhead transcription factors. *Mol Cell Biol* 2002; **22**(7): 2025-36.

19. Zhang J, Cho SJ, Shu L, et al. Translational repression of p53 by RNPC1, a p53 target overexpressed in lymphomas. *Genes Dev* 2011; **25**(14): 1528-43.

20. Wallach T, Schellenberg K, Maier B, et al. Dynamic circadian protein-protein interaction networks predict temporal organization of cellular functions. *PLoS Genet* 2013; **9**(3): e1003398.

21. Talloczy Z, Martinez J, Joset D, et al. Methamphetamine inhibits antigen processing, presentation, and phagocytosis. *PLoS Pathog* 2008; **4**(2): e28.

22. Nair MP, Samikkannu T. Differential regulation of neurotoxin in HIV clades: role of cocaine and methamphetamine. *Curr HIV Res* 2012; **10**(5): 429-34.

23. Patterson TL, Semple SJ, Staines H, et al. Prevalence and correlates of HIV infection among female sex workers in 2 Mexico-US border cities. *J Infect Dis* 2008; **197**(5): 728-32.

24. Jing L, Li JX. Trace amine-associated receptor 1: A promising target for the treatment of psychostimulant addiction. *Eur J Pharmacol* 2015; **761**: 345-52.

25. Asif-Malik A, Hoener MC, Canales JJ. Interaction Between the Trace Amine-Associated Receptor 1 and the Dopamine D2 Receptor Controls Cocaine's Neurochemical Actions. *Sci Rep* 2017; **7**(1): 13901.

26. Pei Y, Asif-Malik A, Hoener M, Canales JJ. A partial trace amine-associated receptor 1 agonist exhibits properties consistent with a methamphetamine substitution treatment. *Addict Biol* 2017; **22**(5): 1246-56.

27. Panas MW, Xie Z, Panas HN, Hoener MC, Vallender EJ, Miller GM. Trace amine associated receptor 1 signaling in activated lymphocytes. *J Neuroimmune Pharmacol* 2012; **7**(4): 866-76.

28. Xie Z, Miller GM. Trace amine-associated receptor 1 is a modulator of the dopamine transporter. *J Pharmacol Exp Ther* 2007; **321**(1): 128-36.

29. Ruocco MR, Chen X, Ambrosino C, et al. Regulation of HIV-1 long terminal repeats by interaction of C/EBP(NF-IL6) and NF-kappaB/Rel transcription factors. *J Biol Chem* 1996; **271**(37): 22479-86.

30. Marcondes MC, Flynn C, Watry DD, Zandonatti M, Fox HS. Methamphetamine increases brain viral load and activates natural killer cells in simian immunodeficiency virus-infected monkeys. *Am J Pathol* 2010; **177**(1): 355-61.

31. In SW, Son EW, Rhee DK, Pyo S. Methamphetamine administration produces immunomodulation in mice. *J Toxicol Environ Health A* 2005; **68**(23-24): 2133-45.

32. Peerzada H, Gandhi JA, Guimaraes AJ, Nosanchuk JD, Martinez LR. Methamphetamine administration modifies leukocyte proliferation and cytokine production in murine tissues. *Immunobiology* 2013; **218**(8): 1063-8.

33. Yu Q, Zhang D, Walston M, Zhang J, Liu Y, Watson RR. Chronic methamphetamine exposure alters immune function in normal and retrovirus-infected mice. *Int Immunopharmacol* 2002; **2**(7): 951-62.

34. Comings DE, Muhleman D, Ahn C, Gysin R, Flanagan SD. The dopamine D2 receptor gene: a genetic risk factor in substance abuse. *Drug Alcohol Depend* 1994; **34**(3): 175-80.

35. Hong CJ, Cheng CY, Shu LR, Yang CY, Tsai SJ. Association study of the dopamine and serotonin transporter genetic polymorphisms and methamphetamine abuse in Chinese males. *J Neural Transm (Vienna)* 2003; **110**(4): 345-51.

36. Liu HC, Lin SK, Liu SK, et al. DAT polymorphism and diverse clinical manifestations in methamphetamine abusers. *Psychiatr Genet* 2004; **14**(1): 33-7.

37. Ikeda M, Okahisa Y, Aleksic B, et al. Evidence for shared genetic risk between methamphetamine-induced psychosis and schizophrenia. *Neuropsychopharmacology* 2013; **38**(10): 1864-70.

38. Uhl GR, Drgon T, Liu QR, et al. Genome-wide association for methamphetamine dependence: convergent results from 2 samples. *Arch Gen Psychiatry* 2008; **65**(3): 345-55.

39. Hart AB, Engelhardt BE, Wardle MC, et al. Genome-wide association study of d-amphetamine response in healthy volunteers identifies putative associations, including cadherin 13 (CDH13). *PLoS One* 2012; **7**(8): e42646.

40. Nicolae DL, Gamazon E, Zhang W, Duan S, Dolan ME, Cox NJ. Trait-associated SNPs are more likely to be eQTLs: annotation to enhance discovery from GWAS. *PLoS Genet* 2010; **6**(4): e1000888.

41. Breen MS, Uhlmann A, Nday CM, et al. Candidate gene networks and blood biomarkers of methamphetamine-associated psychosis: an integrative RNA-sequencing report. *Transl Psychiatry* 2016; **6**: e802.

42. Li MD, Wang J, Niu T, et al. Transcriptome profiling and pathway analysis of genes expressed differentially in participants with or without a positive response to topiramate treatment for methamphetamine addiction. *BMC Med Genomics* 2014; **7**: 65.

43. Nicolae DL, Gamazon E, Zhang W, Duan S, Dolan ME, Cox NJ. Trait-associated SNPs are more likely to be eQTLs: annotation to enhance discovery from GWAS. *PLoS Genet* 2010; **6**(4): e1000888.

44. Zakharchenko O, Greenwood C, Lewandowska A, Hellman U, Alldridge L, Souchelnytskyi S. Meta-data analysis as a strategy to evaluate individual and common features of proteomic changes in breast cancer. *Cancer Genomics Proteomics* 2011; **8**(1): 1-14.

45. Consortium EP, Birney E, Stamatoyannopoulos JA, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* 2007; **447**(7146): 799-816.

46. Roadmap Epigenomics C, Kundaje A, Meuleman W, et al. Integrative analysis of 111 reference human epigenomes. *Nature* 2015; **518**(7539): 317-30.

47. Consortium GT. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* 2013; **45**(6): 580-5.

48. Reback CJ, Larkins S, Shoptaw S. Methamphetamine abuse as a barrier to HIV medication adherence among gay and bisexual men. *AIDS Care* 2003; **15**(6): 775-85.

49. Cachay ER, Moini N, Kosakovsky Pond SL, et al. Active methamphetamine use is associated with transmitted drug resistance to non-nucleoside reverse transcriptase inhibitors in individuals with HIV infection of unknown duration. *Open AIDS J* 2007; **1**: 5-10.

50. Colfax GN, Vittinghoff E, Grant R, Lum P, Spotts G, Hecht FM. Frequent methamphetamine use is associated with primary non-nucleoside reverse transcriptase inhibitor resistance. *AIDS* 2007; **21**(2): 239-41.

51. Plankey MW, Ostrow DG, Stall R, et al. The relationship between methamphetamine and popper use and risk of HIV seroconversion in the multicenter AIDS cohort study. *J Acquir Immune Defic Syndr* 2007; **45**(1): 85-92.

52. Massanella M, Gianella S, Schrier R, et al. Methamphetamine Use in HIV-infected Individuals Affects T-cell Function and Viral Outcome during Suppressive Antiretroviral Therapy. *Sci Rep* 2015; **5**: 13179.

53. Neuhaus J, Jacobs DR, Jr., Baker JV, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J Infect Dis* 2010; **201**(12): 1788-95.

54. Hunt PW, Lee SA, Siedner MJ. Immunologic Biomarkers, Morbidity, and Mortality in Treated HIV Infection. *J Infect Dis* 2016; **214 Suppl 2**: S44-50.

55. Hunt PW, Sinclair E, Rodriguez B, et al. Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection. *The Journal of Infectious Diseases* 2014; **210**(8): 1228-38.

56. Tenorio AR, Zheng Y, Bosch RJ, et al. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. *The Journal of Infectious Diseases* 2014; **210**(8): 1248-59.

57. Lee SL, Byakwaga H, Boum Y, et al. Immunologic Pathways that Predict Mortality in HIV-Infected Ugandans Initiating ART. *The Journal of Infectious Diseases* 2017; **In Review**.

58. Hunt PW. HIV and aging: emerging research issues. *Current opinion in HIV and AIDS* 2014; **9**(4): 302-8.

59. Legarth RA, Ahlstrom MG, Kronborg G, et al. Long-Term Mortality in HIV-Infected Individuals 50 Years or Older: A Nationwide, Population-Based Cohort Study. *J Acquir Immune Defic Syndr* 2016; **71**(2): 213-8.

60. FDA Drug Packaging Insert Methamphetamine Hydrochloride Tablets. https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/005378s034lbl.pdf.

61. Oliveto AH, McCance-Katz E, Singha A, Hameedi F, Kosten TR. Effects of d-amphetamine and caffeine in humans under a cocaine discrimination procedure. *Behav Pharmacol* 1998; **9**(3): 207-17.

62. Stoops WW, Vansickel AR, Lile JA, Rush CR. Acute d-amphetamine pretreatment does not alter stimulant self-administration in humans. *Pharmacol Biochem Behav* 2007; **87**(1): 20-9.

63. Stoops WW, Glaser PE, Fillmore MT, Rush CR. Reinforcing, subject-rated, performance and physiological effects of methylphenidate and d-amphetamine in stimulant abusing humans. *J Psychopharmacol* 2004; **18**(4): 534-43.

64. Kumar AM, Borodowsky I, Fernandez B, Gonzalez L, Kumar M. Human immunodeficiency virus type 1 RNA Levels in different regions of human brain: quantification using real-time reverse transcriptase-polymerase chain reaction. *J Neurovirol* 2007; **13**(3): 210-24.

65. Cockerham LR, Yukl SA, Harvill K, et al. A Randomized Controlled Trial of Lisinopril to Decrease Lymphoid Fibrosis in Antiretroviral-Treated, HIV-infected Individuals. *Pathog Immun* 2017; **2**(3): 310-34.

66. Vandergeeten C, Fromentin R, Merlini E, et al. Cross-clade ultrasensitive PCR-based assays to measure HIV persistence in large-cohort studies. *Journal of Virology* 2014; **88**(21): 12385-96.

67. Thoren KL, Colby JM, Shugarts SB, Wu AH, Lynch KL. Comparison of Information-Dependent Acquisition on a Tandem Quadrupole TOF vs a Triple Quadrupole Linear Ion Trap Mass Spectrometer for Broad-Spectrum Drug Screening. *Clin Chem* 2016; **62**(1): 170-8.

68. Huang Y, Gandhi M, Greenblatt RM, Gee W, Lin ET, Messenkoff N. Sensitive analysis of anti-HIV drugs, efavirenz, lopinavir and ritonavir, in human hair by liquid chromatography coupled with tandem mass spectrometry. *Rapid Commun Mass Spectrom* 2008; **22**(21): 3401-9.

69. Huang Y, Yang Q, Yoon K, et al. Microanalysis of the antiretroviral nevirapine in human hair from HIV-infected patients by liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem* 2011; **401**(6): 1923-33.

70. Baxi SM, Liu A, Bacchetti P, et al. Comparing the novel method of assessing PrEP adherence/exposure using hair samples to other pharmacologic and traditional measures. *J Acquir Immune Defic Syndr* 2015; **68**(1): 13-20.

71. Han E, Lee S, In S, et al. Relationship between methamphetamine use history and segmental hair analysis findings of MA users. *Forensic Sci Int* 2015; **254**: 59-67.

72. Suwannachom N, Thananchai T, Junkuy A, O'Brien TE, Sribanditmongkol P. Duration of detection of methamphetamine in hair after abstinence. *Forensic Sci Int* 2015; **254**: 80-6.

73. Berry MD. Mammalian central nervous system trace amines. Pharmacologic amphetamines, physiologic neuromodulators. *J Neurochem* 2004; **90**(2): 257-71.

74. Elliott JH, McMahon JH, Chang CC, et al. Short-term administration of disulfiram for reversal of latent HIV infection: a phase 2 dose-escalation study. *Lancet HIV* 2015; **2**(12): e520-9.

75. Ingenuity Pathway Analysis (Qiagen). https://apps.ingenuity.com.

76. Kyoto Encyclopedia of Genes adn Genomes Database. http://www.genome.jp/kegg/pathway.html.

77. Molecular Signatures Gene Set Enrichment Analysis Database v6.1 (Broad Institute). http://software.broadinstitute.org/gsea/msigdb.

78. Park J, Munagala I, Xu H, et al. Interferon signature in the blood in inflammatory common variable immune deficiency. *PLoS One* 2013; **8**(9): e74893.

79. Dannenfelser R, Clark NR, Ma'ayan A. Genes2FANs: connecting genes through functional association networks. *BMC Bioinformatics* 2012; **13**: 156.

80. Saito R, Smoot ME, Ono K, et al. A travel guide to Cytoscape plugins. *Nat Methods* 2012; **9**(11): 1069-76.

81. Ma'ayan A. Insights into the organization of biochemical regulatory networks using graph theory analyses. *The Journal of biological chemistry* 2009; **284**(9): 5451-5.