

PROTOCOL: Cannabis, HIV and Mental Processing Systems (CHAMPS)

VERSION: 7 (4/30/2025)

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A Introduction

A1 Study Abstract

This proposal employs novel methods to identify key determinants and consequences of concurrent HIV infection and regular cannabis use. We will acquire extensive phenotype data from peripheral and brain markers of immune activation, brain structure, and neuropsychological performance (NP) in persons living with HIV (PLWH) receiving combination anti-retroviral therapy (cART) (80 regular cannabis users and 80 non-users) and HIV uninfected (HIV-) controls (80 regular cannabis users and 80 non-users). This proposal will provide key insights into the effects of regular cannabis and HIV on peripheral and brain markers of immune function and NP in PLWH and HIV- controls. These insights are critical for cure strategies and ongoing HIV treatment initiatives.

A2 Primary Hypothesis

Our overall hypothesis is that cannabis use leads to increases in inflammation in the peripheral and brain compartments. We also hypothesize that phenotypic signatures due to regular cannabis use and HIV will be delineated through NP and brain volumetrics.

B Background

B1 Prior Literature and Studies

B1.1 Clinical management of persons living with HIV (PLWH) is complex in the combination antiretroviral therapy (cART) era.¹⁻⁴ The majority of PLWH in the US have access to cART, with the goal of having 90% of all PLWH receiving cART and 90% of these individuals achieving viral suppression.⁵ For virally suppressed PLWH on cART, a remaining challenge is reducing persistent immune activity in the periphery and brain. Increases in immune activity in virally suppressed PLWH may be due to multiple mechanisms including cannabis.⁶

B1.2 Cannabis is the most commonly used drug by PLWH.⁷ In the US, most states have legalized either medicinal or recreational cannabis.⁸ Cannabis use is disproportionately higher among PLWH. The estimated prevalence of cannabis use in PLWH ranges from 20 -60%,⁹ with nearly half reporting regular use (operationalized as ≥ 4 times/week).¹⁰ Many PLWH report pre-infection cannabis use that continues after seroconversion to ameliorate HIV symptoms and/or side effects associated with treatment (e.g. neuropathic pain, nausea, mood problems, appetite, and weight loss).¹¹⁻¹³ While cannabis use disorder (CUD), as defined by the Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DSM-5),¹⁴⁻¹⁶ has been linked to worse cART adherence¹⁷ and an increased risk for becoming infected,¹⁸ this may not be the entire story. Central to the current proposal, a previous analysis revealed that progressive cognitive worsening among PLWH on cART was evident only in individuals with substance use (e.g. cannabis).¹⁹

B1.3 Cognitive dysfunction persists in PLWH despite cART. Approximately 40% (12%–99%) of PLWH exhibit neurocognitive symptoms despite sustained viral suppression with cART.⁴⁰ Chronic cognitive difficulties following cART result from a combination of risk factors, including irreversible neurologic injury prior to treatment onset, persistent HIV reservoirs, immune activation, and inflammation.²⁰⁻²⁵ *A central tenet of the current proposal is that these mechanisms are potentiated by regular use of drugs of abuse, such as cannabis. Confirmation of this hypothesis would be clinically significant because cannabis use is a modifiable risk factor that can be addressed through clinical interventions, but rigorous science is required to guide policy and clinical practice.*^{17, 26}

B1.4 Chronic immune activity and inflammation exists in the brain and periphery despite cART. HIV persists in resting memory CD4 T-cells and other long-lived cellular reservoirs, including macrophages in the brain,²⁷ and can cause chronic brain inflammation.²⁸⁻³⁰ In PLWH with prolonged viral suppression, infection of the brain is mostly by macrophages and microglia, with ongoing low-level viral replication and brain inflammation.³¹⁻³³ Brain autopsy studies have detected residual inflammatory monocytes in PLWH who had prolonged viral suppression with cART.³⁴ These findings suggest that latently infected myeloid cells and chronic peripheral inflammation are not prevented by adequate adherence to cART. *A greater understanding of the role of residual inflammation in the periphery and brain is needed to inform HIV cure strategies.*^{27, 35}

B1.5 Cannabis use affects inflammation in PLWH. Cannabis contains tetrahydrocannabinol (THC) and cannabidiol (CBD) which bind to cannabinoid (CB) receptors CB1 and CB2, respectively.³⁶ CB1 receptors are expressed in the frontal cortex, hippocampus, basal ganglia, and cerebellum and account for its psychoactive effects. Regular cannabis use downregulates CB1 receptors³⁷ and is linked to decreased protection against oxidative stress.³⁸ This is particularly relevant for HIV, as oxidative stress has been proposed to be involved in the pathogenesis of cognitive dysfunction.³⁹ Therefore, it is possible that regular cannabis use increases susceptibility to neuro-inflammation in PLWH.⁴⁰ Indeed, initial evidence suggests that cannabis use in PLWH is associated with metabolic brain changes, particularly in the temporal lobe and prefrontal cortex.⁴¹ This finding is consistent with the spatial distribution of the CB1 receptor within the brain. CB2 receptors are predominantly expressed on immune cells and astrocytes.^{7, 42-44} *In vitro* CB2 activation of CD4+ T cells reduces HIV replication.⁴⁵ However, the opposite effect is seen in macrophages, which continue to display high rates of infection in the brain.⁴⁵

Within the periphery, discrepancies have been observed regarding the effects of cannabis use on inflammation. Within HIV- controls, the combination of cannabis use and alcohol was associated with elevated monocyte activation, as measured by plasma sCD163.⁴⁶ PLWH on cART who were cannabis users had increased inflammatory monocytes and pro-inflammatory cytokine secretion, all of which affect HIV disease progression.⁴⁷ In another study of PLWH, regular cannabis users had increased sCD14 in the periphery compared to PLWH who did not use cannabis. In contrast, a recent study showed that PLWH who used cannabis had reduced inflammation in the periphery (IL-16, c-reactive protein (CRP), and soluble tumor necrosis factor receptor II (sTNFRII)).⁴⁸ A major limitation of this recent study was the lack of female participants (only three of thirty-five individuals). This is important because the density of cannabinoid receptor in the brain differs by sex, with a higher density reported in males compared to females.⁴⁹ Understanding sex as a biological variable is a clinical and research priority. *As noted in recent reviews there is a pressing need for additional research aimed at understanding the differential effects of cannabis exposure (THC, CBD, and other metabolites) on peripheral and brain inflammation within PLWH.*^{9, 50}

B1.6 Controversy exists regarding the effects of cannabis use on cognition.^{51, 52} In cross-sectional studies of HIV- individuals, the acute effects of cannabis on cognitive test performance included reduced memory function. However, it remains unclear if these effects are transient or chronic in nature.^{51, 53, 54} A recent meta-analysis did not observe long-term effects of cannabis use on cognition, but the authors noted that most studies had significant design flaws (e.g. not including mood disorders or not accounting for poly substance use).⁵⁵ Additionally, cannabis metabolites have often not been measured.⁵⁶ Results from the few

longitudinal studies of HIV- individuals conducted to date, indicate that regular cannabis use is associated with chronic cognitive symptoms that persist months after individuals have achieved sustained abstinence.⁵⁷

In PLWH, cross sectional studies of cognitive performance have shown mixed results. Some studies demonstrated lower performances on tests of learning, memory, and attention among PLWH who were cannabis users compared to HIV- controls,⁵⁸⁻⁶⁴ while others observed no differences^{54, 55} and one study reported better cognitive performance among individuals who reported recent cannabis use.⁶⁵ However, these studies did not utilize similar methods to ascertain cannabis use patterns or evaluate cognitive performance. Further, these studies did not measure ratios of THC versus CBD.^{9, 66}

B1.7 Few structural neuroimaging studies have investigated the effects of HIV and cannabis use.

Anatomical magnetic resonance imaging (MRI) studies in HIV- controls have consistently observed that regular cannabis users have smaller hippocampal volumes, a region rich in CB1 receptors.⁶⁷ However, morphological changes in other brain regions including the orbitofrontal cortex, striatum, and amygdala, have shown mixed results.^{68, 69} To date, only one study has examined the combined effects of cannabis use and HIV on brain structure in adults.⁶¹ Greater exposure to cannabis was associated with reduced brain volumes in the entorhinal and fusiform cortices, but no interaction or additive effects were observed between cannabis use and HIV. However, this study had relatively few HIV- individuals and traditional measures of cognitive performance were not examined. Additionally, 50% of the sample was not virologically controlled and the concentrations of THC, CBD, and other metabolites were not measured. While we have recently used diffusion basis spectrum imaging (DBSI) to non-invasively measure brain inflammation (cellularity) in PLWH,⁷⁰ this technique has not been utilized with regards to cannabis use.

B1.8 The proposed study has high clinical relevance. Understanding the interplay of cannabis and HIV is a critical research gap that will be filled by this application. Our central hypothesis is that regular cannabis use, especially in PLWH results in immune dysregulation in both the periphery and the brain⁷¹⁻⁷³ (**Figure 1**). We will rigorously evaluate if regular cannabis use increases peripheral and brain inflammation and is associated with reductions in brain structure and cognition. Observed effects of cannabis use will be modulated by concentrations of THC and CBD metabolites with high exposure to THC associated with more deleterious effects on both cognitive and volumetric measures. These results could transform cannabis public policies for PLWH and lead to substantial changes in recommendations for PLWH in the clinic as HIV is a qualifying condition for legal medical cannabis.

B1.9 Specific gaps that will be addressed include: (1) Determining the relative contribution of THC versus CBD exposure to brain inflammation and integrity metrics using a validated, high-throughput, sensitive, and specific assay that measures 11 cannabinoids and metabolites. (2) Quantifying the effects of THC versus CBD exposure on peripheral inflammatory biomarkers. Animal and *in vitro* studies demonstrate that activation of endocannabinoids influence inflammatory cytokines and chemokines that may further contribute to HIV-associated neurotoxicity.⁷⁴ However, these studies need to be performed in PLWH who regularly use cannabis. (3) Employing cutting-edge multimodal neuroimaging and advanced analytics to identify the factors that explain and predict chronic effects of regular cannabis use.

B1.10 This proposal addresses high priorities identified by the National Institute of Health (NIH) Office of AIDS Research (OAR) strategic plan with regards to HIV-associated co-morbidities, co-infections, and complications. *This proposal addresses OAR's priorities by examining neurological complications of HIV, mental illness, and cannabis use across the lifespan. Our aims focus on the effects of regular cannabis use on peripheral and brain markers in PLWH and HIV- controls.* This proposal: (1) Increases our understanding regarding the etiology, pathogenesis, spread, and persistence of HIV/AIDS among people who use cannabis. To address this priority, our proposal will assess the effects of regular cannabis use on HIV infection, inflammation and immune activity, and brain integrity. Approximately 50% of PLWH followed at the Washington University in Saint Louis (WUSTL) Infectious Disease (ID) clinic regularly use cannabis. Our proposal will evaluate the effects of the quantity, frequency, and type of

cannabis used on inflammatory mechanisms and immune health in PLWH. We hypothesize that there will be multifaceted responses between peripheral and brain inflammation based on cannabinoid receptor distributions and functions.

(2) Evaluates the effects of drug use (cannabis) and improves health outcomes among PLWH who do use drugs. We will elucidate mechanisms by which cannabis use affects clinical outcomes in HIV (neurobehavioral phenotypes and neurological comorbidities). We will assess the effects of regular cannabis use and HIV on peripheral and brain markers of immune activity, cognition, and brain structure in PLWH and HIV- controls. These insights are critical for cure strategies and ongoing HIV treatment initiatives. If cannabis is found to be deleterious, it is a potentially modifiable factor through counseling paradigms.

(3) Accelerates scientific discoveries in HIV/AIDS and substance use research by enhancing the pace of translational processes through team science. This proposal brings together scientists and clinicians with expertise in substance use and HIV. Our multi-disciplinary team has been at the forefront of evaluating peripheral and brain inflammation, quantifying cannabis use, and utilizing cognitive measures and neuroimaging methods in PLWH and HIV- controls. The team is composed of experts in neuroimaging (Dr. Ances), blood and cerebrospinal fluid (CSF) inflammatory markers (Dr. Burdo), neurobehavioral phenotyping of HIV (Dr. Paul), cannabis use (Dr. Gilman), and statistics (Dr. Vaida). Each investigator has an ongoing record of accomplishments and has helped advance their respective field. The team has continued to have weekly meetings via Zoom despite COVID-19 and crossing multiple time zones. A multi-PI plan is in place for Drs. Ances and Burdo, who have complementary and integrated expertise and have extensively worked together.

Using a cross sectional study design, we will acquire phenotype data from peripheral and brain markers of immune function (e.g. CD4/CD8 ratio, monocyte subpopulations, plasma sCD14 and sCD163, CSF MCP-1 and neopterin, and DBSI cellularity), brain structure (volumetrics), and cognition from four groups: virally controlled (< 20 copies/mL) PLWH on cART with regular cannabis use (PLWH, CB+; n = 80), virally controlled (< 20 copies/mL) PLWH with no cannabis use (PLWH, CB-; n = 80), HIV- controls with regular cannabis use (CON, CB+; n = 80) and HIV- controls with no cannabis use (CON, CB-; n = 80)

C Study Objectives

C1 Primary Aim

Specific Aim 1: Cannabis use increases peripheral and brain immune activation and inflammation indices in virally controlled PLWH on cART.

Hypothesis 1a: Peripheral and brain immune activity (e.g. inversion of CD4/CD8 ratio, elevated sCD14, sCD163, monocyte subpopulations, MCP-1, neopterin, and cellularity) are elevated in PLWH, CB+ compared to other groups (PLWH, CB-; CON, CB+; and CON, CB-).

Hypothesis 1b: Peripheral and brain immune indices (e.g. CD4/CD8 ratio, sCD14, sCD163, monocyte subpopulations, MCP-1, neopterin, and cellularity) will be moderated by concentrations of THC and CBD metabolites.

Hypothesis 1c: Data driven classification models will reveal novel mechanisms and associations between regular cannabis use and peripheral and brain immune activity indices (e.g. CD4/CD8 ratio, sCD14, sCD163, MCP-1, neopterin, and monocyte subpopulations, and cellularity) that differentiate HIV serostatus from cannabis use.

C2 Secondary Aim

Specific Aim 2. Cannabis use impairs cognitive performance and brain structure in both PLWH/CB+ and CON/CB+.

Hypothesis 2a: Cognitive performance (particularly working memory and executive function) and brain volumetrics (temporal and parietal lobes) will be worse in regular cannabis users regardless of HIV status.

Hypothesis 2b: The effects of cannabis on brain integrity will be moderated by THC and CBD concentrations.

Hypothesis 2c: Data-driven classification models will reveal novel highly dimensional mechanisms and associations between cannabis use and brain integrity that differentiate HIV serostatus from cannabis use.

C3 Innovation and Rationale for the Selection of Outcome Measures

3. Innovation

C3.1 Quantitative evaluation of both peripheral and brain markers of immune activity and inflammation will improve our understanding of the effects of regular cannabis use in PLWH. We will evaluate the effects of regular cannabis use on validated plasma and CSF markers of immune health and inflammation. This proposal will be the first to simultaneously acquire extensive phenotype data from peripheral and brain markers of immune function (e.g., CD4/CD8 ratio, peripheral monocyte subsets, plasma sCD14, and sCD163 and CSF MCP-1 and neopterin), brain structure (volumetrics), brain inflammation (cellularity), and cognition (neuropsychological performance (NP) testing) in virally controlled PLWH on cART and HIV- controls. Immune responses will be examined with regards to duration, frequency, type, and amount of cannabis used.

C3.2 Novel neuroimaging methods of neuro-inflammation in PLWH will be used. Microscopic barriers (e.g., cellular and nuclear membranes and myelination) in the brain constrain the free movement of water molecules, resulting in apparent diffusivity measured by diffusion tensor imaging (DTI).⁷⁵ Although DTI has been performed in PLWH,^{76, 77} this technique provides a single averaged diffusion profile but cannot differentiate multiple microstructural components—including brain inflammation.⁷⁸ Within any given brain voxel, there may be varying amounts of normal and damaged axons that are myelinated or demyelinated,⁷⁹ as well as varying amounts of cells (microglia and macrophages) and free space.⁷⁰ Diffusion basis spectrum imaging (DBSI) allows us to distinguish these compartments and differentiate normal anatomy from pathology due to inflammation.

C3.3 Cellularity measurements derived from DBSI correlate with brain microglia/macrophage accumulation. Within an animal model of brain inflammation, immunohistochemistry findings from a control group and a 4-week cuprizone-treated group were compared based on cellularity measurements derived from DBSI.⁸⁰ Cuprizone treatment led to an increase in the number of activated microglia and macrophages that entered the brain. Strong correlations were seen between cellularity (derived from DBSI) and the number of cells (microglia and macrophages). This technique has been successfully translated to multiple diseases in humans.⁸⁰⁻⁸³ *This study will be the first to use DBSI to study the effects of regular cannabis use on brain inflammation in virally controlled PLWH and HIV- controls.*

Dr. Ances has significant expertise in applying novel neuroimaging techniques to PLWH.⁸⁴⁻⁸⁷ The DBSI technique was initially developed at WUSTL, and Dr. Ances and his team were the first to utilize it in PLWH.⁷⁰ As noted above, DBSI will non-invasively provide new insights into brain inflammation at high resolution compared to existing techniques that require administering a contrast agent or injecting a

radioactive isotope using positron emission tomography (PET). The DBSI sequence and analyses are approved by the FDA and are standard on most clinical MRI scanners. Furthermore, the MRI sequences proposed can be easily acquired on routine 3T MRI scanners that are used by the AIDS Clinical Trials Group (ACTG).

C3.4. Urine metabolites of cannabis will be compared to neuroimaging and cognitive indices. From urine, we will assay 11 cannabinoids and metabolites including: THC, 11-hydroxy- Δ^9 -tetrahydrocannabinol (11OH-THC), 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid (THC-COOH), 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid glucuronide (THC-C-gluc), cannabinol (CBN), cannabidiol (CBD), cannabigerol (CBG), cannabidivarin (CBDV), Δ^9 -tetrahydrocannabivarin (THCV), and 11-nor-9-carboxy- Δ^9 -tetrahydrocannabivarin (THCV-COOH). *This study will be the first to compare urine cannabinoids and metabolites to neuropsychological performance and imaging measures of neuroinflammation (DBSI) in virally controlled PLWH and HIV- controls.* This is important because many studies only assess frequency of cannabis use, and it is now understood that potency, not just frequency, is an important indicator of exposure.⁸⁸

C3.5 Data-driven techniques will be applied to identify novel interactions. Prior studies of cannabis use and HIV have relied on traditional statistical methods that are generally insensitive to nonlinear models and high-order interactions believed to underlie complex clinical phenotypes. Our team has significant expertise in applying data driven techniques to discover novel mechanisms of changes in brain integrity within PLWH.^{89, 90} Biomarkers of peripheral and brain inflammation, detailed brain volumetrics, cognitive scores, demographics, and other relevant clinical data will be evaluated using data driven machine learning algorithms. *This study will be the first to use machine learning based feature selection methods to identify latent relationships and conditional dependencies seen with regular cannabis use and HIV status.* These methods are applicable for high dimensional data with binary or continuous outcomes, do not depend on heuristics, are noise-tolerant, and are sensitive to feature interactions and non-linear relationships, making them the optimal choice for the identification of novel biomarkers. Moreover, the methods proposed in this application will allow us to evaluate brain integrity without being biased by *a priori* regions of interest.

D Study Design

D1 Overview or Design Summary

A total of 160 PLWH (80 regular cannabis users and 80 non-users) and 160 demographically similar healthy HIV- controls (80 regular cannabis users and 80 non-users) may undergo multimodal neuroimaging, immunophenotyping (including blood and cerebrospinal fluid), and neuropsychological testing to address the specific aims. PLWH may be recruited from WUSTL Infectious Disease (ID) Clinic, the AIDS Clinical Trial Unit (ACTU), Volunteers for Health, The Spot, other area providers and by hanging flyers at various places in the community. HIV- controls may be recruited from similar demographic communities as the PLWH, as well as Volunteers for Health and the surrounding community and social organizations.

D2 Subject Selection and Withdrawal

D2.1 Inclusion Criteria

(1) Participants must be 18–70 years old.

- (2) PLWH must have documented HIV infection for approximately 1 year, be on a cART regimen for approximately ≥ 3 months, and be virally well-controlled (< 200 copies/mL).
- (3) HIV- controls must have confirmed HIV- serostatus.
- (4) Participants must complete at least 9 years of education.
- (5) Participants must be able to provide informed consent.
- (6) Female participants must have a negative pregnancy test or written documentation of tubal ligation, hysterectomy, post-menopausal status, etc. and cannot be breastfeeding.
- (7) Participants should have no contraindications to an MRI scan.
- (8) Participants should be willing to have cannabis use history confirmed via clinical interview and urine analysis.
- (9) All inclusion criteria at PI discretion.

D2.2 Exclusion Criteria

- (1) History of neurological disorder (e.g., stroke, epilepsy, head injury with loss of consciousness for > 5 minutes, developmental learning disability, etc.).
- (2) Uncontrolled major affective disorder; any history of schizophrenia. Major depression or Bipolar disorder controlled with no hospitalizations and suicidal ideation in the past year is allowed.
- (3) Current substance use disorder (SUD) other than cannabis use disorder (CUD) and mild Alcohol Use Disorder as defined by DSM-5 criteria. A past SUD approximately > 1 year before the time of study enrolment may be allowed.
- (4) currently prescribed anti-coagulants (only exclusionary if participant will engage in optional LP).
- (5) an allergy to lidocaine or similar anesthetic (only exclusionary if participant will engage in optional LP).
- (6) a history of a bleeding disorder (only exclusionary if participant will engage in optional LP).
- (7) claustrophobia or device or hardware that would prevent magnetic resonance imaging (MRI) scanning.
- (8) all exclusion criteria at PI discretion.

D3 Subject Recruitment Plans and Consent Process

D3.1 Recruitment:

Drawing on previous experience recruiting and maintaining a large cohort of PLWH at the WUSTL ACTU and WUSTL ID Clinic, we understand the importance of fostering and maintaining an atmosphere of trust and cooperation among community leaders and agencies serving this group. Accordingly, the WUSTL ACTU and WUSTL ID Clinic have established and maintained close alliances with HIV healthcare and community organizations. We will continue to draw upon these strong relationships in our recruitment efforts of PLWH in the St. Louis area. Targeted educational and

recruitment in-services may be conducted with WUSTL and community providers to ensure awareness of this project and to assist in identifying eligible participants. Flyers will also be hung throughout the metro area at bus stops, churches, medical clinics, marijuana dispensaries, etc., as well as posted on our lab website and other social media. Additionally, we have established strong relationships of trust with individuals who have participated in our previous studies.

If a PLWH meets preliminary eligibility criteria, key providers may initiate discussions regarding possible participation in this study. Once the study is introduced to the individual and (s)he expresses interest in participating, the provider may give the patient the necessary information to contact study staff themselves, as well as a copy of the Informed Consent document for their review. Provider may also give participant's contact information to study staff to initiate contact. If, for any reason, the patient indicates disinterest (either to the referring provider or later to study staff), no further contact will be made unless initiated by the patient.

Study staff may review medical records before contacting participants to check for gross eligibility and potential participants will complete an IRB-approved phone screen to ascertain interest and confirm general inclusion/exclusion requirements. Qualifying individuals who are interested in participation will then undergo a phone screening, followed by a more comprehensive screening during an in-person visit.

Once a person is deemed eligible, individuals will be given a more detailed description of the study including possible risks and benefits, either over the phone or in person. As part of the screening process, a medical record review may also be completed to fill in and confirm documented HIV+ status and length of time on cART and viral suppression (for PLWH); and presence of any medical condition or other criterion that would meet exclusion criteria or make it unsafe to participate in the study.

HIV negative healthy controls that are demographically matched as much as possible will be recruited from the friends, family and partners of their HIV+ counterparts, as well as from various community organizations as described above.

Based on our previous experience, we believe approximately 1,200 participants may need to be initially screened by phone to reach a sample size of 320 individuals. Before signing consent or undergoing study procedures a clinician or study staff will evaluate participants for acute intoxication. If acute intoxication is suspected the participant may be rescheduled. At the on-site visit the following will be obtained: (1) informed consent; (2) urine sample for drug screen, urine cannabinoids and metabolites, and pregnancy test (for all pre-menopausal female participants without documentation of tubal ligation, hysterectomy, or menopause); (3) oral HIV test (for HIV- controls only); (4) medical record review; (5) assessment of mental health and performance of activities of daily living; (6) multi-dimensional assessment of substance use; and (7) MRI metal screening form. The urine sample will be tested using the Rapid Drug Screen (RDS). The RDS detects cannabis, amphetamines, cocaine, opiates, barbiturates, methamphetamines, benzodiazepines, and phencyclidine. Any discrepancies amongst any dimensions of substance use may be brought to case conference for consensus diagnosis. If the participant is not severely depressed (BDI-II < 29), has a negative urine screen other than THC, and has no MRI contraindications; then s/he may be enrolled. Based on our previous experience we believe approximately 30 individuals may be ineligible due to depression scores or non-cannabis substance use, leading to the final sample size of 320 participants. After consent and completion of assessments above, each enrolled study participant may receive the following procedures during this on-site visit (~6 hours): a neuromedical examination, serum laboratory tests, neuropsychological performance testing, questionnaires, and neuroimaging. A follow-up lumbar puncture (LP) may be scheduled ~ 1 week or more after the primary visit.

We have found that it is especially important to try to accommodate our participants' requests regarding their study visit. The assessment schedule designed for this proposal has the necessary flexibility to adapt to the real-life complexities of working with clinical populations. Through flexible scheduling, barrier mitigation, and positive feedback, this study minimizes the extent to which a participant's daily life issues adversely impact their ability to complete scheduled visits. Reducing

participant burden will also be a priority through scheduling study and clinic appointments on the same day whenever possible and desired by the participant. Overcoming barriers such as lack of transportation is being addressed through the request of specific funds for transportation costs to ensure that financial difficulties do not impede a participant's ability to complete assessments. If a participant misses the scheduled visit, we may contact him/her and obtain reasons that contributed to the missed visit. A planned future visit will take this information into account and accommodations will be made whenever possible.

Our previous experience has also highlighted the importance of employing a proactive retention program that maintains accurate, up-to-date contact information in real time. This has been accomplished by encouraging participants to let us know when their contact information changes but also requires periodic checks on the accuracy of records. Regular outreach with periodic greeting cards and calls may be used to maintain ties with the participants, acknowledging their ongoing importance as collaborators in our research efforts. Additionally, the PI and his team have offices in the same building as the WUSTL ACTU and WUSTL ID Clinic. Our team has successfully used this close proximity to ensure study participant engagement whenever an individual has a clinic visit.

D3.2 Informed Consent:

The cornerstone of protection in research is informed consent. All key personnel involved in the design and conduct of the research involving human participants will receive the required education on the protection of human research participants (CITI) prior to funding of this project. Procedures to recruit participants for the protocol and obtain their informed consent will be conducted and supervised by investigators involved in this proposal. Written informed consent will be obtained from all participants before any study procedures are initiated. We request a *partial* waiver of consent in order to review records before a participant consents in order to reduce burden. No other study procedures will occur without the participant having provided written informed consent.

The consent form, which incorporates HIPAA authorization, contains a description of the purpose and procedures, risks, procedures to minimize them, and possible benefits. Participants will be given sufficient time to consider their participation, encouraged to discuss their enrollment with friends or significant others, and assured that they are free to withdraw consent at any time and that discontinuing participation in this study will not prejudice their current or future medical care. The objectives of the project, all of the requirements for participation, and any possible discomforts and risks will be clearly explained at each contact to the participants orally and in writing in lay terms which they are able to comprehend. All participants will sign an informed consent form, approved by the WUSTL institutional review board (IRB), before they can participate in the study. The capacity to consent will be determined by the principal investigator or delegate. By interviewing the patient, we will determine whether the affected individual understands the central elements of the study procedures. Patients with capacity to consent will be invited to participate by the physician-investigator or designee. Only after a participant has reviewed and expressed understanding of the IRB-approved consent, and had all questions answered, written informed consent will be obtained. Once a written informed consent has been provided, study staff will continue to review what to expect in the next study visit when we talk with participants over the phone and prior to all procedures. Our ongoing engagement in this process provides additional assurance that all of our participants will be truly informed and able to consent throughout all steps of the study. If at any time a participant declines to participate and withdraws consent, they will immediately be withdrawn from the study at their request.

In order to minimize participants' and staff's exposure alike, whenever possible, consent, neuropsychological assessments and questionnaires may be completed over the phone and/or through Zoom instead of in person/recording answers on paper. For questionnaires, participants may still be sent a link to the survey in Redcap as is our current practice, but if they are unable to access it, the questions may be asked over the phone or in person as study staff record the responses directly into Redcap. If neuropsychological assessments and questionnaires are completed in person, whenever possible staff will

encourage social distancing by placing the participant in one room and following the previous remote procedures from a different room in the same building.

D3.4 Risks and Benefits

Potential risks:

There are potential risks associated with some of the procedures in this study. However, the procedures have been planned by the investigators to minimize the danger of any major complication and have successfully been used in multiple previous studies done by these researchers. As with any study that collects sensitive information, breach of confidentiality is a potential risk, however, all study personnel will undergo training specific to the use of human participants in research (CITI), will be Health Insurance Portability and Accountability Act (HIPAA)-trained, and will be approved by the Institutional Review Board (IRB) to be engaged in the study. Only participant identification numbers (instead of PHI) will be used for analyses, and all data will be maintained according to the WUSTL School of Medicine and HIPAA “two lock” policy (i.e., locked data cabinets found in locked offices, password protected documents on password protected computers located in locked offices, etc.). No one other than study personnel will have access to this data.

Risks associated with neuropsychological performance testing and questionnaires:

Likely: None

Less likely: Participants may experience fatigue, mental and/or emotional distress as a result of the questions that are asked during this testing. If a particular question makes a participant feel uncomfortable, s/he may discuss its importance with the specially trained interviewer. S/he may also choose not to answer any question with which s/he still feels uncomfortable.

Rare: None

Risks associated with magnetic resonance imaging (MRI):

Participants will be asked to inform study staff if they have any of the following: heart rhythm disturbances, cardiac pacemaker, aneurysm clip(s), implanted insulin/drug pump, neurostimulator (TENS unit), biostimulator/bone growth stimulator, hearing aid/cochlear implant, Gianturco coil (embolus coil), vascular clip(s), surgical clip(s) or staple(s), heart valve prosthesis, Greenfield vena cava filter, middle ear implant, penile prosthesis, shrapnel or bullet, wire sutures, tattooed eyeliner, any type of dental item held in place by a magnet, any other implanted item not mentioned, diaphragm/intrauterine device (IUD), intraventricular shunt, wire mesh, artificial limb or joint, any orthopedic item (i.e., pins, rods, screws, nails, clips, plates, wire, etc.), dentures, dental braces or any other type of removable dental items, or pregnancy.

Participants will be screened by MRI certified staff, including evaluation of metallic or electronic objects in or on their person, medical implants, tattoos, etc. using screening forms and workflows to reduce the risks listed below. If the participant experiences any the symptoms listed below and does not wish to continue, the study will be stopped immediately.

- Serious risk of injury from metallic objects pulled by the force of the magnet - For this reason, all persons entering the MRI environment must undergo standardized screening procedures;
- Serious risk of damage to electronic devices - The MRI staff will assess whether the research protocols are compatible with the FDA labeling of devices;

- Serious risk of burns - To reduce this risk, participants with tattoos or non-removable body piercings may need to undergo additional screening. Also, to reduce the risk of burns, participants are asked to change into clothing provided by the imaging facility;
- Low risk of tissue heating, with a possible rise in core body temperature;
- Low risk of hearing damage due to the loud noise of the MRI - Ear protection is required during the MRI scan;
- A low risk that participants may experience claustrophobia, which may manifest as anxiety, dizziness, or lightheadedness;
- Low risk of temporary muscle stiffness associated with lying still, which may be worse in patients with pre-existing arthritis;
- Low risk of peripheral nerve stimulation, which commonly manifests as muscle spasms.
- Rare risk that participants may experience a sensation of flashing lights while in the scanner.

Likely: If a participant has metal implants in his/her body (such as a pacemaker, or metal pin, or shavings, etc.) s/he may be at risk when close to the machine. Participants could also experience claustrophobia (anxiety or nervousness while inside small spaces) when in the scanner, as well as stiffness from lying still for an extended period of time.

Less likely: The scanner produces a loud repetitive knocking noise during the study that some people find bothersome.

Rare: Occasionally, some people may experience a short period of dizziness or feel faint after being in the scanner. There is also a rare possibility that a serious abnormality of which the participant is unaware may be discovered during the scan.

Risks associated with optional lumbar puncture:

Likely: Pain at the site of needle insertion.

Less Likely: Headache.

Rare: Lowered blood pressure, infection, leaking of spinal fluid after the procedure, nerve injury, and bleeding at the needle insertion site. Additionally, an allergic reaction to the ultrasound gel, antiseptic (e.g. iodine) or the numbing medicine (e.g. lidocaine) used during the spinal tap could include itching, hives, swelling, shortness of breath, difficulty breathing, changes in blood pressure and heart rhythm, loss of consciousness or, in a rare case, death.

Following the procedure, the most common complaint is a headache. Up to 5 in 100 people who have a lumbar puncture get a headache that lasts 1-2 days (if fluoroscopy is used, up to 3 in 100 people may get a headache). Headaches that last more than 1-2 days or headaches that are of severe intensity may be due to a leak of the cerebrospinal fluid from the spinal space where the needle was inserted. A leak such as this can be treated with a blood patch. Rare or very uncommon complaints include low blood pressure and dizziness, bleeding into the spinal canal, or an infection of the cerebrospinal fluid (known as meningitis). These rare complaints could be serious.

Risks associated with blood draw:

Likely: Pain, stinging, bruising and bleeding at the site of needle insertion.

Less Likely: None

Rare: Infection at the site or blood clot. Additionally, an allergic reaction to the ultrasound gel, if the Butterfly device is used during the blood draw, could include itching, hives, swelling, shortness of breath, difficulty breathing, changes in blood pressure and heart rhythm, loss of consciousness or, in a rare case, death.

Risks associated with oral HIV test:

Likely: None

Less Likely: None

Rare: There is always a risk of participants finding out for the first time that they are HIV positive when it was previously believed they were negative. This could cause some emotional distress, but the study physicians and /or a mental health clinician or designee will be available to talk with participants regarding the ramifications and treatment options of this diagnosis, as well as to offer support should the individual become upset upon hearing their status. Individuals will be referred to treatment and on-site case management before they leave.

All data will be safeguarded in accordance with HIPAA and the principles and practices of strict^[1] confidentiality. Studies are done for research purposes only. The risks of breaching confidentiality will be rigorously limited by the use of locked and restricted access to data. No identifiers will be included in any reports generated by this study. All key personnel involved in the design or conduct of research that involves contact with human subjects will receive the required education on the protection of human research subjects prior to funding of this project.

The risks to subjects from the neuroimaging, NP evaluations and medical interview are minimal.^[1] Occasionally, the neurological examination or neuropsychological performance tests may induce anxiety or concern. This is usually controllable with appropriate counseling and explanation and ultimately by reminding subjects that the study is completely voluntary and that they are under no obligation whatsoever to participate. The risks of magnetic resonance imaging, specifically the danger of scanning an individual with metal in/on their person, will be virtually eliminated by having all subjects complete a metal screening sheet prior to their scans and by review of medical records to search for any confounds. Occasionally individuals experience anxiety and claustrophobic feelings during the scan procedure. Appropriate explanation is usually sufficient to allay anxiety, but again, volunteers will be reminded that they are free to stop the study at any time.

Only highly trained research staff, nurses, and physicians will be utilized to collect data. These^[1] individuals will be experts in confidential and professional interaction with study subjects. Subjects will be notified in the informed consent document that any serious suicidal or homicidal information obtained will be shared as necessary with appropriate authorities to protect the life of the subject. If a subject is found to be suicidal or homicidal during any evaluation, the individual performing the evaluation may take immediate suicide and homicide precautions, contacting the PI, study physician, subject's primary physician and any authorities necessary to ensure safety. If the subject is the sole adult household member and suicide/homicide is not deemed to be imminent, precautions and referrals, including emergency room contacts, will be provided. If any subject is deemed to be imminently suicidal and/or homicidal, emergency response will be contacted as soon as possible. Subjects will also be told in the informed consent document that research staff may provide a request for a referral for professional care as necessary. To protect against any misuse of knowledge about study participation, the informed consent document will advise potential subjects that employers or insurers could act negatively if they learned of participation in this study. Subjects may choose not to disclose their participation to these entities.

If there is a clinically meaningful abnormality identified or other medical need for sharing of the standard clinical MRI portion of the exam, workflows have been established for sharing the images and reports. Any potentially clinically meaningful abnormal findings are communicated to the participant by the Principal Investigator or a designee and, if the participant permits, to their physician. If requested for

clinical use, and following hospital HIPAA policies, a copy of the participant's MRI scan and report can be uploaded into the participant's electronic health record (EHR). Once the MRI is part of the EHR, then participants may request additional copies to be sent to additional physicians using a standard medical record request. Lab results within normal limits and other measures not requiring follow-up may not be provided to the participant or their healthcare providers, as these procedures are being done as part of a research protocol and not intended to diagnose or treat any underlying medical condition.

E Study Procedures

E1 Screening for Eligibility

Pre-consent screening (done through review of medical records and interview with participant) to insure participants DO meet the following criteria:

- (1) Participants must be 18–70 years old.
- (2) PLWH must have documented HIV infection for approximately 1 year, be on a cART regimen for approximately ≥ 3 months, and be virally well-controlled (< 200 copies/mL).
- (3) HIV- controls must have confirmed HIV- serostatus.
- (4) Participants must complete at least 9 years of education.
- (5) Participants must be able to provide informed consent.
- (6) Female participants must have a negative pregnancy test or written documentation of tubal ligation, hysterectomy, post-menopausal status, etc. and cannot be breastfeeding.
- (7) Participants should have no contraindications to an MRI scan.
- (8) Participants should be willing to have cannabis use history confirmed via clinical interview and urine analysis.
- (9) All inclusion criteria at PI discretion.

And do NOT meet the following criteria:

- (1) History of neurological disorder (e.g., stroke, epilepsy, head injury with loss of consciousness for > 5 minutes, developmental learning disability, etc.).
- (2) Uncontrolled major affective disorder; any history of schizophrenia. Major Depression or Bipolar Disorder, controlled with no hospitalizations or ER visits related to any psychiatric disorder/SI in approximately the past year is allowed.
- (3) Current substance use disorder (SUD) other than cannabis use disorder (CUD) and/or mild Alcohol Use Disorder as defined by DSM-5 criteria. A past SUD approximately > 1 year before the time of study enrolment may be allowed.
- (4) currently prescribed anti-coagulants (only exclusionary if participant will engage in optional LP).

- (5) an allergy to lidocaine or similar anesthetic (only exclusionary if participant will engage in optional LP).
- (6) a history of a bleeding disorder (only exclusionary if participant will engage in optional LP).
- (7) claustrophobia or device or hardware that would prevent magnetic resonance imaging (MRI) scanning.
- (8) all exclusion criteria at PI discretion.

Review of Medical Records (to verify subjects' responses to pre-screening questions and make sure meet inclusion/exclusion criteria). Medical records, including but not limited to HIV/AIDS-related outpatient visits, inpatient hospitalizations, blood/diagnostic/laboratory/imaging tests, substance use/abuse/dependence, mental health treatment, and cardiovascular history/events/surgeries may be obtained from participants' health care providers. Additional information may also be obtained from clinical interviews or questionnaires found in participants' records related to their medical care. The names/contact information/DOB of these individuals may also be collected. ^[L]^[SEP] The research team may also review the subject's research records from other WUSM studies. This will allow the research team to determine/clarify eligibility. ^[L]^[SEP] Also, results of additional labs necessary for the VACS Index (CD4, VL, HCV, AST, ALT, hemoglobin, platelets, fibrinogen-4, creatinine and GFR) and to complete the Charlson Comorbidity Index may be collected.

E2 Schedule of Measurements

Visit 1: (may be split over 2 days or whatever creates the least burden for the participant)

At the first onsite visit the following will be obtained:

- (1) informed consent
- (2) urine sample for drug screen, urine cannabinoids and metabolites, and pregnancy test for all female participants without written documentation of tubal ligation, hysterectomy, menopause, etc.
- (3) oral HIV test (for HIV- controls only)
- (4) medical record review (may also be done at time of phone screen)
- (5) MRI screening form
- (6) Beck Depression Inventory 2nd Edition (BDI-II)
- (7) Hopkins Verbal Learning Test & Recall [HVLT]
- (8) Locator form (*address and 9-digit zip code may be used to analyze social determinants of health*)
- (9) Scanning Cover Sheet (*demographics, medical history*)

If the participant is not impaired and has no MRI contraindications, then s/he may be enrolled. After consent and completion of assessments above, each enrolled study participant may receive the following procedures:

- (10) MRI
- (11) Neuro-medical Examination
- (12) Vitals and BMI
- (13) blood & CSF laboratory tests (if there is difficulty locating a reliable vein or the LP interspace, a Butterfly Network hand-held portable ultrasound device may be used):
 - MCP-1 (CSF)
 - Neopterin (CSF)
 - PlasmaSCD14

- Plasma^SCD163
- Plasma^IL-10
- Plasma^IL-1ra
- CD14-CD16+ monocytes
- CD14+CD16+ monocytes
- CD14+CD16- monocytes
- CD4
- CD8
- CD4:CD8 ratios
- VL
- CBC w/differential
- CMP
- Lipid profile
- HbA1c
- Assays to acquire VACS index
- any additional labs found to be relevant per the discretion of the PI's

(14) Neuropsychological performance testing which involve a series of written, verbal, and motor tasks requiring the participant to follow instructions, remember words and numbers, draw objects, and complete timed actions.

- Trails A&B
- Logical Memory I and II
- WAIS-IV Digit Symbol
- Grooved Pegboard
- Symbol Search
- WAIS-IV Letter Number Sequencing
- Wide Range Achievement Test, 4th Edition [WRAT]
- D-KEFS Color-Word Interference Test – Trials 1, 2, and 3
- WAIS-IV Digit Span Forward and Backward
- Brief Visuospatial Memory Test [BVM-T-R]
- Verbal Fluency [F, A, S]
- Category Fluency (Action Naming Test) – Action Fluency
- Animal Fluency
- Timed Gait

(15) Questionnaires:

- Early Life Stress (ELS)
- Kreek-McHugh-Schluger-Kellogg (KMSK) scale
- Timeline Follow Back Method Assessment (TLFB calendar)
- Cannabis Use Disorder Identification Test - Revised (CUDIT-R)
- Marijuana Effect Expectancy Questionnaire (MEEQ & MMEEQ)
- Marijuana Motives Measure
- DSM-5 CUD checklist & DSM-5 substance checklist (all participants will complete the CUD checklist, but the substance checklist will only be completed on individuals who have used that substance >10 times in the past year).
- Substance Social Disruption Questions
- Hospital Anxiety and Depression Scale (HADS), anxiety subset only
- Marin Apathy Scale
- 12-item Short Form Health Survey (SF-12)

- Pittsburgh Sleep Quality Index (PSQI)
- Charlson Comorbidity Index (found on Scanning Cover Sheet)
- Activities of Daily Living (ADL's) – Lawton & Brody
- UCLA Loneliness Scale

Due to the length of time these assessments may take, we may recommend to participants that the session be split into 2 visits. At the participant's request/for scheduling purposes, however, the assessments may all be done on one day or split into more than 2 visits. Questionnaires may be administered on paper or electronically on a secure computer. Secure links to the questionnaires in RedCap may also be emailed to participants at their request to reduce their burden and allow them complete at home.

The following privacy protections may be enacted for all email communications involving PHI; 1) a test email may be sent to the participant to verify their identify (confirm correct recipient) and that this email may be sent in a secure manner (i.e., [secure] in subject line); 2) the body of the email may instruct the participant to send all information as a response to this thread and to not remove the "[secure]" from the subject line; 3) we will document in the research record the participant's agreement to provide information over email.

A follow-up lumbar puncture (LP) may be scheduled ~ 1 week to 6 months after the last study visit.

Visit 2 – Approximately 1 week to 6 months after the initial visit, participants may be scheduled for a lumbar puncture.

The physician performing the LP will explain all potential risks and benefits, review contraindications and potential complications of the procedures, answer all of the participant's questions, and obtain verbal consent before beginning; written informed consent will have already been obtained at the first visit. A brief H&P may be performed as necessary. Participant's vitals may be taken before and after the procedure. Physician will then open the LP tray without compromising sterility and obtain any additional supplies that may be needed (i.e., spinal needles, extra tubes, etc.).

Patient may be positioned in either lateral decubitus/fetal position or sitting upright leaning forward over a small table or LP chair or as directed by physician.

LP interspace will be located, skin will be aseptically cleaned using the skin prep provided in the LP tray, and the area appropriately draped.

Approximately 10 mL of 1% or 2% lidocaine may be injected into the appropriate area. Spinal needle will be inserted and up to 25 cc of cerebrospinal fluid (CSF) will be collected.

When complete, the patient will be allowed to recline or sit in the exam room until the physician deems it safe for discharge. Instructions will be given to drink extra fluids and not lift anything heavy over the next 24 hours.

Following the LP, tubes of CSF will be labeled and prepped/delivered to the appropriate lab/location. Physician will complete note documenting the procedure, location of interspace used (i.e. L2-L3, L3-L4, etc.), number of attempts, total amount of CSF collected, and any complications that arose during the procedure.

E3 Safety and Adverse Events

E3.1 Protection Against Risk

Serious adverse events will be reported to the IRB within the timeline outlined in their policies and procedures.

All data will be safeguarded in accordance with HIPAA and the principles and practices of strict confidentiality. Studies are done for research purposes only. The risks of breaching confidentiality will be rigorously limited by the use of locked and restricted access to data. No identifiers will be included in any data analyses or reports generated by this study. All key personnel involved in the design or conduct of research that involves contact with human participants will receive the required education on the protection of human research participants prior to funding of this project.

The risks to participants from the neuroimaging, neuropsychological performance (NP) evaluations and neuromedical examination are minimal. Occasionally, the neurological examination or neuropsychological performance tests may induce anxiety or concern. This is usually controllable with appropriate counseling and explanation and ultimately, by reminding participants that the study is completely voluntary and that they are under no obligation whatsoever to participate. The risks of magnetic resonance imaging, specifically the danger of scanning an individual with metal in/on their person, will be virtually eliminated by having all participants complete a metal screening sheet prior to their scans and by review of medical records to search for any confounds. Appropriate explanation is usually sufficient to allay anxiety, but again, volunteers will be reminded that they are free to stop any portion of the study at any time.

The LP will be done under aseptic conditions by a trained clinician, such as Dr. Ances. Any risks of LPs or blood draws will be minimized by the use of staff trained and proficient in the use of aseptic techniques and standard universal precautions. If participants experience any irritation of the skin, an alternative cleansing agent or ultrasound gel will be used. If the irritation is more than mild, the participant will be offered the option of discontinuing that portion of the study.

It is possible that a volunteer for the HIV- group may test positive when his or her status was believed to be negative. In that case, the participant may be counseled and referred to the appropriate clinic (Linkage to Care, etc.), on-site case management, the WUSTL ID clinic, and/or to his or her primary care physician for further counseling and treatment options. If the participant is in severe emotional distress as a result of this testing, it may be arranged for him/her to speak with Dr. Ances or other physician immediately or go to ER. All participants will be reminded that the oral HIV test is a screening tool that could offer false positive or false negative results, especially if the person has been exposed to the virus in the past three months, and must be confirmed by ELISA/Western Blot before definitively stating that the person has HIV positive or negative. It is for this reason that we are not obligated to report the test results to any public health entities.

Only highly trained research staff, nurses, and physicians will be utilized to collect data. These individuals will be experts in confidential and professional interaction with study participants. Participants will be notified in the informed consent document that any serious suicidal or homicidal information obtained may be shared as necessary with appropriate authorities to protect the life of the participant. If a participant is found to be suicidal or homicidal during the evaluation, the individual performing the evaluation may take immediate suicide and homicide precautions, contacting the PI, study physician, participant's primary physician and any authorities necessary to ensure safety. If any participant is deemed to be imminently suicidal and/or homicidal, 911 may be contacted as soon as possible; otherwise research staff will provide a referral for professional care as necessary. To protect against any misuse of knowledge about study participation, the informed consent document will advise potential participants that employers or insurers could act negatively if they learned of participation in this study. Participants may choose not to disclose their participation to these entities.

E3.2 Safety and Compliance Monitoring

Data and Safety Monitoring Plan (DSMP)

Serious adverse events may be reported to the IRB within the timeline outlined in their policies and procedures. The stopping criteria and guidelines to be used for this protocol may include the following: 1) The study may be stopped if there is clear evidence of harm or harmful side effects of the procedures used in this protocol; 2) In the event that a serious adverse event (SAE) occurs that increases the risks to participants, the study may be stopped and an investigation be conducted and a findings report generated by the PI; 3) Should there be an SAE or adverse event (AE) that occurs at a frequency greater than 5%, it will be added to the consent document, if not already addressed, and enrollment may be halted while a determination is made regarding the potential risks to participants. The risks of the study are low, however, the PI and study physicians as well as the WUSTL IRB will review safety material and the protocol at least annually.

F Statistical Plan

Considerations for data science methods: Continuous variables will be transformed as needed prior to analyses to improve normality and linearity of the relationship to the designated outcome variable. Raw NP tests that measure time on a task will use an inverse transformation $f(t) = 1/t$, measuring the speed of task completion; for other tests an empirical normal quantile transformation and normative corrections for demographics will be considered, which will lead to scale scores and z-scores. NP global and sub-domain values are likely to be normally distributed, which will allow for multivariable linear regression. The distributions of depression, apathy, and HRQoL scores are likely to be right-skewed and over-dispersed; therefore, negative binomial regression may be considered for analyses. All statistical tests will be two-sided, with significance declared at the $p < 0.05$ level (unless otherwise stated). In addition to hypothesis testing, 95% confidence intervals (95%CI) will be determined for effect sizes of interest. Sex will be investigated as a potential predictor of interest for each hypothesis and analysis. In exploratory analyses, correction for multiple comparisons will preserve the false discovery rate (FDR) at the 0.05 level.

Demographics: Demographic, medical, psychosocial, and lab characteristics of participants will be summarized using N (%) for categorical variables, and mean, standard deviation, and range for numeric variables.

Cannabis: The effect of regular cannabis use will be examined primarily using the grouping of individuals into cannabis users and non-users. Additional exploratory analyses will quantify exposure (KMSK and urine metabolites) and will examine the relationship between outcomes of interest and cannabis metabolites among cannabis users. Both linear and non-linear relationships, including quadratic and smooth effects via generalized additive models, will be evaluated.

Composite peripheral inflammatory response: The primary inflammatory response will be a composite, continuous variable, summarizing the level of a compartment at the individual level. The composite variable will be the first principal component (PC1) of the set of peripheral inflammatory markers (CD4/CD8 ratio, sCD14, sCD163, and monocyte subpopulations: CD14-CD16+, CD14+CD16+, CD14+CD16-) obtained via principal component analysis (PCA). Individual biomarkers will be log-transformed and standardized prior to the transformation. This amounts to standardizing each log-transformed marker to a standard deviation of 1 and creating a single composite marker, which accounts for multivariate correlations.

Composite brain inflammatory response: A composite brain inflammatory response will be created using the first principal component of a PCA transformation of CSF inflammatory markers (MCP-1 and neopterin) and cellularity derived from DBSI.

Aim 1: *Hypothesis 1a.* The effect of regular cannabis use on peripheral and brain inflammatory markers (primary response: composite markers; secondary: individual inflammatory markers) will be analyzed using linear models (analysis of covariance), including as predictors cannabis status, HIV, and their interaction. If an interaction effect between cannabis and HIV status is not significant, additive effects will be evaluated. Demographic factors such as Wide Range Achievement Test (WRAT)-4, sex, and age will be examined as explanatory variables and incorporated into the final models if significant at $p < 0.20$ on backward model selection. Secondary marker analyses will control for multiple tests by keeping the overall FDR at level $\alpha = 0.05$. The effect of cannabis on peripheral and brain inflammatory markers among PLWH and HIV- controls will be reported based on these models using a 95%CI. In the absence of a HIV-by-cannabis interaction, the additive effects of cannabis and HIV are of primary interest. If an interaction is present, then the primary comparison will be between CB+ and CB- groups among PLWH (at $\alpha = 0.05$). Secondary analyses will compare the PLWH, CB+ to the other two groups (at familywise error rate $\alpha = 0.05$).

Hypothesis 1b. The relationship between cannabis exposure (KMSK and urine analysis of cannabis metabolites) and peripheral and brain immune activity markers (primary response: composite markers; secondary: individual markers, possibly log-transformed, as noted above) will be analyzed among regular cannabis users using linear models. Demographic factors such as the WRAT-4, sex, and age will be examined as explanatory variables and incorporated into the final models if significant at $p < 0.20$ on backward model selection. In addition to linear effects, non-linear cannabis effects will also be considered, including quadratic effects and smooth effects fitted via generalized additive models. Possible interactive effects between cannabis exposure levels and HIV will also be examined.

Hypothesis 1c: Data-driven feature selection methods⁹²⁻⁹⁵ will be performed to identify novel mechanisms and associations between regular cannabis use and peripheral and brain immune activity measures that differentiate HIV serostatus from cannabis use. The analysis will yield a subset of biomarkers that are most indicative of the effects of cannabis on brain immune activation and inflammation, while excluding irrelevant and redundant biomarkers. Continuous variables will be normalized to zero mean and unit variance. Features will be scaled independently to a common interval to ensure that features with higher magnitudes are not given preference during training. All analysis will be conducted with 10-fold cross validation with 10 repeats to ensure the reliability of the results.⁹⁶ Individual models will be trained with a categorical cross entropy loss function, and evaluated based on sensitivity, specificity, and receiver operator curve (ROC) area under the curve (AUC) F1 analyses. Models will be compared using mean misclassification rate and Bayesian information criteria.⁹⁷ Analysis will be performed in common programming languages and packages (MATLAB, Python, and R).

Power Analysis for Aim 1:

Hypothesis 1a. With the proposed sample size of $n = 320$, we have $\geq 80\%$ statistical power to detect an HIV-by-cannabis group interaction effect (difference-in-differences) corresponding to Cohen's $d = 0.634$ and $d = 0.758$, for peripheral and brain immune activation markers, respectively. In absence of this interaction, we have 80% power to detect a cannabis effect among PLWH of Cohen's $d = 0.446$ for peripheral and $d = 0.533$ for brain immune activity markers. In secondary analyses, we have 80% power to detect a difference between PLWH, CB+ group and either the CON, CB+ or CON, CB- groups of Cohen's $d = 0.491$ or $d = 0.587$ (periphery/brain respectively), Holm-Bonferroni corrected. These calculations assume a 70% LP completion rate, per our past experience. This sample size is sufficient for the planned analyses. In our preliminary analyses we found an effect size of $d = 0.52$ for the PLWH comparison of CB+ and CB- (Figure 3A).

Hypothesis 1b. We have $\geq 80\%$ power to detect an association between cannabis exposure and peripheral immune activity markers explaining $R^2 = 0.047$ or more of the variance in the response, corresponding to a small Pearson correlation coefficient of $r = 0.219$ or higher. This effect size is achievable. In our preliminary data, we observed higher correlation levels (Figure 4). For brain inflammatory activity

markers the corresponding values are $R^2=0.069$, $r=0.262$. The power calculations used statistical software (G*Power).

Hypothesis 1c: Sample size determination for classifier development aims will seek to improve predictor accuracy and significance.⁹⁸ Sample size determinations were conducted using proposed published methods. Training and testing errors of cross validated progressively sampled models will be assumed to follow power laws and will be evaluated based on asymptotic error value convergence to identify appropriate sample sizes.^{99, 100} Results will be optimized based on nonlinear weighted least squares fitting.¹⁰¹ Cross validated model performance will be compared using empirical and uniform priors to determine the effect of balanced classes.¹⁰² Cross validation of data with randomly permuted labels will be used to identify the significance of model results.¹⁰³

Aim 2:

Hypothesis 2a: The effect of cannabis and HIV on cognition will be quantified through linear models, similar to those discussed under Hypothesis 1a, adjusting for confounding factors. The main NP outcomes of interest are raw scores from the working memory/executive function domain scores. Secondary outcomes are cognitive domain-specific scores and global deficit score, with FDR control, as discussed above. We expect to find additive but not interactive effects of HIV and cannabis use on primary NP outcomes. Separate analyses will examine the effect of cannabis on NP outcomes, controlling for peripheral immune indices.

Analyses of brain volumetrics will focus on the hippocampus as the primary outcome. Secondary outcomes will include the caudate, amygdala, putamen, and pre-frontal regions (with FDR corrections). These volumetric analyses will follow a similar pattern to that described for NP outcomes.

Hypothesis 2b: The effect of THC and CBD concentrations among cannabis users on NP and brain volumetrics will be analyzed using linear models. Demographic factors such as the WRAT-4, sex, and age will be examined as explanatory variables and will be incorporated into the final models if significant at $p<0.20$ in backward model selection. Possible non-linear cannabis effects will be explored, including quadratic effects and smooth effects fitted via generalized additive models. A possible interaction between cannabis exposure levels and HIV will also be examined.

Hypothesis 2c: Data-driven feature selection methods⁹²⁻⁹⁵ will be performed to reveal highly dimensional mechanisms and associations between cannabis use and brain integrity (cognition and volumetrics) that differentiate HIV serostatus from regular cannabis use. The analysis will yield a subset of biomarkers that are most indicative of the effects of cannabis on brain integrity (NP and volumetrics), while excluding irrelevant and redundant biomarkers. Continuous variables will be normalized to zero mean and unit variance. Features will be scaled independently to a common interval to ensure that features with higher magnitudes are not given preference during training. All analysis will be conducted with 10-fold cross validation with 10 repeats to ensure the reliability of the results.⁹⁶ Individual models will be trained with a categorical cross entropy loss function, and evaluated based on sensitivity, specificity, and receiver operator curve (ROC) area under the curve (AUC) F1 analyses. Models will be compared using the mean misclassification rate and Bayesian information criteria.⁹⁷ All analysis will be performed in common programming languages and packages (MATLAB, Python, and R).

Power Analysis for Aim 2:

Hypothesis 2a: With the proposed sample size of $n=320$ we have $\geq 80\%$ statistical power to detect additive effects of HIV status and cannabis use on NP and volumetrics outcomes corresponding to Cohen's $d=0.314$ each. This effect size is consistent with those determined in our preliminary data (Cohen's $d=0.584$ for cannabis and $d=0.418$ for HIV in *Figure 5*).

Hypothesis 2b: We have $\geq 80\%$ power to detect an effect of cannabis exposure among cannabis users on NP or volumetric outcomes corresponding to $R^2=0.047$ proportion of variance explained, or equivalently

a Pearson correlation coefficient of 0.219. For comparison, in our preliminary analyses we found $r=0.23$ (Figure 3B).

Hypothesis 2c: Sample size determination for classifier development aims will seek to improve predictor accuracy and significance.⁹⁸ Sample size determinants will be conducted using proposed published methods. Training and testing errors of cross validated progressively sampled models will be assumed to follow power laws and will be evaluated based on asymptotic error value convergence to identify appropriate sample sizes.^{99, 100} Results will be optimized based on nonlinear weighted least squares fitting.¹⁰¹ Cross validated model performance will be compared using empirical and uniform priors to determine the effect of balanced classes.¹⁰² Cross validation of data with randomly permuted labels will be used to identify the significance of model results.¹⁰³

G Data Handling and Record Keeping

1. Confidentiality and Security

All data will be safeguarded in accordance with HIPAA and the principles and practices of strict confidentiality. Studies are done for research purposes only. The risks of breaching confidentiality will be rigorously limited by the use of locked and restricted access to data. No identifiers will be included in any reports generated by this study. All key personnel involved in the design or conduct of research that involves contact with human subjects will receive the required education on the protection of human research subjects prior to beginning work on this project. All electronic data transferred to Drs. Vaida Burdo, or Paul will be de-identified and sent via encrypted email, encrypted hard drive by courier, or by providing them access to a secure database (i.e. RedCap, CNDA, WUSTL Box, etc.). All biological data sent to Temple University will be done via courier.

2. Training

All study staff must complete CITI and HIPAA training and be listed as engaged with the IRB before they will be able to perform any study procedures. Study staff must also review protocol and sign a delegation log expressing understanding and commitment to adhere to it.

3. Case Report Forms and Source Documents

Copies of participants' medical records containing data to be used in the study (i.e. lab values, diagnoses, lists of medication, etc.) should be placed in the file whenever possible. Should these source documents not be available, subject report is acceptable.

4. Records Retention

Paper/hard copy records (hard copy surveys, questionnaires, case report forms, etc.) The risks of breaching confidentiality will be strictly limited by the use of locked and restricted access to data, as well as the use of participant id numbers rather than names in the data base. Hard copy research records with identifying information will be kept in a secure environment according to WUSM and HIPAA 2-lock procedure. Medical records containing PHI and research records will be maintained according to WUSM & HIPAA 2-lock policy and procedure. Only coded data will be used for data analysis and publications.

Electronic records (computer files, electronic databases, etc.) - The risks of breaching confidentiality will be strictly limited by the use of locked and restricted access to data. Research Electronic Data Capture (REDCap) is a secure, password-protected, web-based electronic data capture application that is on the Washington University Secure Network. Electronic Records will be created, maintained and stored in Redcap where possible. REDCap is HIPAA compliant and in compliance with university policies. The REDCap database will contain details and identifiers from participants' medical records (name, date of

birth, address, social security number, medical record numbers, dates of procedures, etc.) as well as a participant code number. Only qualified study staff will have access to these and other data and identifiers as needed. This database may be updated on a periodic basis from medical records in the following years in order to acquire more current information on medications, surgeries, cognitive testing, disease activity, and disease history. No identifiers will be included in any reports generated by this study, and all sensitive electronic information containing identifiers will be kept in compliance with WUSM & HIPAA approved “2 lock” policy and practice. All imaging data will be stored on WUSM's CNDA and/or on CD's, external hard drives, servers, etc. secured in the PI's office, NIL, other lab space under the 2-lock rule. All other data will be entered into a secure database system, such as REDCap.

Biologic samples (blood draws, cheek swabs, saliva samples, tissue samples, etc.) – Tubes containing blood samples and cerebrospinal fluid (CSF) tubes will be labeled with the subject's ID code, date of draw, and time of draw. Samples will be transported by engaged study staff to the appropriate processing lab at WUSM before being shipped to Dr. Burdo's lab in batch.

H Study Monitoring, Auditing, and Inspecting

Oversight of the study will be the responsibility of the PI and his delegates, including the study coordinator(s). Internal self-audits for quality assurance purposes may be conducted as necessary to ensure study procedures are being followed and for the integrity of the data. The study will also be reviewed at least annually by HRPO/WU IRB.

I Study Administration

I.1 Funding Source and Conflicts of Interest

Funding is through an R01 through the National Institute of Mental Health (NIMH) at NIH.

I.2 Subject Stipends or Payments

Participants may be remunerated for completing all assessments as follows:

- MRI = \$75
- Neuropsychological assessments = \$60
- Questionnaires = \$15
- Laboratory evaluations = \$25
- Stool sample and dietary survey = \$25
- Lumbar Puncture = \$75

Participants may be remunerated after completing each procedure rather than having to wait until the end of the study to receive their full remuneration. If a participant completes all study procedures, they will be paid a total of \$275.

Remuneration for the neuropsychological assessments may be prorated, based on the per cent of assessments that are completed; for example, if an individual only completes half (50%) of the assessments, they may only receive half of the payment (\$30.00). If a participant does not complete the MRI, laboratory evaluations or lumbar puncture, they may not be paid, however, if they make a good-faith effort and are unable to complete, they may be remunerated.

In our experience, the biggest barrier to study retention has been access to transportation, not untimely or inadequate reimbursement. Consequently, we have included the costs of taxis into our budget and intend to provide these services to all participants upon request. These exact methods and amount of participant remuneration have been employed in multiple studies conducted in our lab over the past ten years.

I.3 Study Timetable

Benchmarks will be established to ensure successful completion of the aims within 5 years. We may recruit participants during all years of the grant. Serum and CSF samples may be batched and sent to Dr. Burdo for analysis. Urine analysis samples may be batched and sent to Dr. Klawitter for analysis. During Year 3, the relationships between peripheral and brain markers of inflammation, NP, and neuroimaging may be evaluated using data driven classification models. Starting in Year 3 results of the study may be written up and in Year 5 an R01 to longitudinally follow these individuals will be submitted.

	Year 1	Year 2	Year 3	Year 4	Year 5
Recruitment of Participants					
Analysis of Peripheral and Brain Inflammatory Data					
Analysis of NP and Neuroimaging Data					
Data Driven Classification Models					
Dissemination of Findings					

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