**Protocol**

**IRB# 201500625**

1. **Project Title**: Alcohol Effects on HIV-Associated Brain Dysfunction

**Short Title: *ARCH II Study*** (Alcohol Research Center on HIV Study II)

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1. **Abstract:**

The proposed study will continue the work conducted in Research Component 1 from the Alcohol Research on HIV (ARCH I) study conducted at Brown University, which involves an investigation of the effects of ethanol (ETOH) consumption on Human Immunodeficiency Virus (HIV)-associated brain dysfunction, incorporating state-of-the-art brain imaging methods along with clinical and laboratory methods to assess the interactive effects of ETOH consumption on HIV-associated brain dysfunction.

1. **Background:**

Mounting evidence suggests that the central nervous system (CNS) effects of HIV worsen as people survive and reach more advanced age with chronic infection. They also experience a variety of comorbid medical conditions, which increase in prevalence and severity due to chronic HIV infection. Psychosocial and neuropsychiatric comorbidities also commonly have adverse effects on functional outcome. Substance abuse and dependence are well recognized examples of this with many past studies showing reduced functional and health outcomes in these individuals. ETOH use once received little clinical attention relative to other drugs of abuse. This is no longer the case given declining rates of intravenous drug use, ETOH remaining an integral part of American culture, and evidence that ETOH exacerbates HIV effects on the brain and other organ systems. Our findings to date show that structural and cerebral metabolic brain alterations occur as a function of the interaction of HIV and ETOH. Cerebral metabolite disturbances that reflect inflammatory pathophysiology, damage to membranes, and neuronal loss are observed on Magnetic Resonance Spectroscopy (MRS), along with volumetric and white matter disturbances, including reduced white matter coherence on Diffusion Tensor Imaging (DTI). As depicted in the adjacent model, we propose that ETOH interacts with host and viral factors to exacerbate HIV-associated brain dysfunction. HIV and ETOH also contribute directly to systemic metabolic disturbances in the blood that correspond with cerebral metabolite

abnormalities on brain MRS.

There are two broad objectives. The first is to continue an ongoing line of research, extending current findings by incorporating functional neuroimaging (FMRI) approaches, along with additional MRS methods that will enable us to delineate both functional and cerebral metabolic disturbances affecting specific functional brain systems that are associated with the interaction of ETOH and HIV, as well as alterations in functional connectivity within and between these systems. The second objective is to examine the extent to which reductions in ETOH consumption among heavy drinkers with HIV infection that result from a motivational intervention lead to improvements in these functional and metabolic neuroimaging measures, as well as neurocognitive performance. This intervention, which is the focus of ARCH Research Component 2, has been shown to be effective in reducing ETOH consumption by 50% over a 6-month follow-up whereas HIV care as usual was associated with no changes in drinking. Recent data from ARCH Research Component 1 indicate that HIV infected patients with heavy ETOH consumption have FMRI abnormalities and exhibit alterations on other neuroimaging measures compared to moderate drinkers and people who do not drink at all. Moderate drinkers did not differ from non-drinkers. Abnormalities of cerebral white matter integrity on DTI were also found among heavy ETOH users. These effects were associated with current ETOH consumption, but not lifetime history, suggesting that ETOH-associated brain abnormalities in people with HIV may be reversible when there has been a significant reduction in ETOH use. Neuroimaging, laboratory, and neurocognitive assessment will be conducted at baseline and at 6-months following randomization to motivational intervention (MI) or assessment only. The findings from this study will provide important information on how heavy ETOH and HIV interact to affect the brain functional responsiveness, and the extent of improvement that might be gained by reducing heavy ETOH use. The study will also likely yield new neuroimaging metrics of functional, metabolic, and structural brain dysfunction, potentially leading to their use as neuroimaging biomarkers for the assessing patients with HIV and other diseases that affect the brain.

1. **Specific Aims:**

The primary focus of this project is the association between heavy drinking and CNS dysfunction in people living with HIV and whether intervention to reduce drinking in heavy drinking HIV-infected participants can improve CNS function. Functional outcome will be measured primarily by neurocognitive indices. Structural brain changes will be measured on T1-weighted MRI and DTI. Brain metabolite levels will be measured with MRS. Laboratory measures (e.g., CD4 count, HIV viral load in plasma) will serve as covariates. A three group design will be used with repeated measures at baseline, 6 months, and 12 months: moderate/minimal drinking (no intervention), heavy drinking (no intervention), and heavy drinking (receive intervention). An equal number of men and women will be recruited. The design will enable testing of alcohol x intervention interactions over 12 months for the neurocognitive, morphometry, DTI, and MRS data and for analysis of how specific biomarkers of disease status, liver function, inflammatory processes, and metabolite levels predict change in neurocognitive function and brain abnormalities over time.

*Aim 1:* Demonstrate worsening neurocognitive and functional/metabolic/structural brain abnormalities among HIV-infected adults who drink heavily as determined by the NIAAA. NIAAA heavy drinking criteria is more than 4 drinks on any day or 14 per week for men and more than 3 drinks on any day or 7 per week for women. **Overall hypothesis**: HIV-infected adults who are heavy ETOH users will have greater neurocognitive and neuroimaging disturbances.

*H1.1.*Greater baseline neurocognitive impairments will exist among heavy drinkers, with most significant deficits related to working memory, processing speed, attention-executive functions, and learning efficiency.

*H1.2.*AbnormalFMRI responses during verbal working memory (n-back) and attention (set switch) and rest will be observed among HIV-infected heavy ETOH users. Specific fMRI effects as a function of ETOH use will include: a) abnormaldefault network activation and deactivation; b) altered functional connectivity between vulnerable brain regions (e.g., frontal-striatal), and c) altered FMRI responses during sustained working memory performance. These effects will reflect a decreased dynamic range of brain response resulting from the interaction of HIV and ETOH use.

*H1.3.* Cerebral metabolite abnormalities on both proton MRS will be greatest among HIV-infected heavy drinkers. MRS abnormalities will correspond with brain ROIs exhibiting fMRI abnormalities. Cerebral MRS and systemic metabolic biomarkers, including pro-inflammatory cytokine/chemokine concentrations, will be strongly associated reflecting common cerebral and systemic pathophysiology.

*H1.4* Cerebral white matter abnormalities, as measured by DTI in the internal capsule, corpus callosum, and frontal and parietal centrum semiovale, will be greatest among HIV-positive heavy drinkers.

*Aim 2:* Demonstrate improved brain function (fMRI), metabolic health (MRS) and cognitive performance among HIV-infected adults who reduce their ETOH consumption.

*H2.1.*Baseline neurocognitive deficits will improve following reductions in ETOH use over six months with effects evident across affected cognitive domains (e.g., speed, working memory, attention-executive, learning).

*H2.2.*Task-related and resting state fMRI abnormalities will improve with reduced ETOH consumption.

*H2.3.* Cerebral metabolite abnormalities (-MRS) will also improve with reduced ETOH consumption, and will correspond with improved systemic biomarkers (e.g., serum cytokine/chemokine concentrations).

*H2.4.* Improvements in DTI metrics of white matter integrity will occur with reduced ETOH consumption in HIV+ heavy drinkers.

*Aim 3:* To determine saliva miRNA profiles by RNA-seq next generation sequencing in subjects enrolled in the NIH-funded Florida Cohort to screen for published plasma-derived miRNA associated with HIV/AIDS progression.

Salivary miRNAs are potential non-invasive diagnostic biomarkers, however the precise salivary miRNA profiles in PLWH are still unknown. This sets up the rationale to employ in depth miRNAseq profiling approach to identify and quantify the differential miRNA profiles in saliva and plasma and evaluate miRNA biomarkers predictive of the disease progression and therapy response.

*Aim 4:* To demonstrate feasibility of minimally processed miRNA saliva assay.

Great research effort is devoted to the development of inexpensive and non-invasive assays for accurate quantification of infectious disease diagnostics and therapy monitoring. MiRNA are very stable and abundant in body fluids, and have been proposed as biomarkers for cancer and other diseases. MiRNA can be easily measured using ddPCR. These considerations set up the rationale for developing a pilot saliva-based miRNA assay**.**

*Secondary aims:* We will examine the extent to which particular comorbidities and clinical factors (age, HCV, HIV-RNA and cART treatment status, other substance abuse mediate: 1) observed brain abnormalities and cognitive impairments among HIV-infected heavy drinkers; and 2) improvements following reduced consumption. Particular emphasis will be placed on age and HIV, with secondary analyses directed at examining how HIV x ETOH interactions change as a function of advanced age, and HCV co-infection.

1. **Research Plan:**
2. ***Participant Recruitment*** - We will recruit up to a total of 214 HIV-infected adults (men = 107; women = 107; age: 25-70 years). These participants will be will be recruited from the Southern HIV Alcohol Research Consortium (SHARC) cohort at the University of Florida, of which Dr. Robert Cook (Co-I) is the director. The SHARC includes HIV-infected adults recruited from several different community populations in Northern Florida. Participants will also be recruited from the North Florida/South Georgia VA through UF’s Consent2Share database. To supplement the number of individuals recruited, participants will also be recruited from the general community through various forms of advertisement. Women will be oversampled to achieve an equal distribution of men and women. All participants will be used to address Aim 1, with comparisons made between the people with heavy ETOH consumption (n = 152) and those without heavy ETOH consumption (n = 62). For Aim 2, only participants with current heavy ETOH (n=152) will be randomized to the MI or no intervention conditions.
3. ***Recruitment Procedures:*** Florida participants, who have consented to be contacted for future research and are part of existing registries developed through the Southeastern HIV-Alcohol Research Consortium SHARC (Florida Site), will be identified, contacted by phone, and screened by SHARC study staff using approved telephone script. No identifying information will be collected during this phone call. Participants in this registry will be asked if they would like to hear about a new research study. Interested participants will then be given more information about the study and a brief telephone screening. Advertisement for the study will also be placed at the VA and throughout the general community, including community health centers. Individuals who respond to the advertisement will be given more information about the study and a brief telephone screening. No identifying information will be collected from the telephone screenings. A team consensus meeting will occur to clarify participant eligibility if necessary. Those who appear eligible based on their responses will be invited to participate in the study and asked to schedule a baseline appointment at University of Florida’s Clinical and Translational Research Building, which would last up to 3 ½ hours. **Consent documents will be reviewed on their first study visit in private, designated study rooms.**

1. ***Inclusion criteria:*** 1) Men and women; 2) Age: 25-70 yrs.; 3) HIV-infected 4) English speaking; 5) Physically mobile; 6) Willingness to participate in the MI to reduce ETOH consumption.

***Exclusion criteria:*** 1) Neurological disorders (e.g., dementia, stroke, seizures, traumatic brain injury). 2) Evidence of dementia (MoCA < 20) 3) Past opportunistic brain infection 4) Current major psychiatric disturbance, including severe intractable major depression or suicidality based on structured clinical interview for DSM-IV (SCID) and CESD. 5) Unstable medical conditions (e.g., cancer); basal cell skin and limited prostate cancer are allowed. 6) MRI contraindications (e.g., pregnancy, claustrophobia, metal implants).7) Physical impairment precluding motor response or lying still.

1. ***Approach:*** This study employs a prospective randomized case-control cohort between group

repeated-measures experimental design. We will contrast HIV-infected adults with current heavy ETOH consumption by NIAAA criteria with HIV-infected adults without heavy ETOH use. Within each of these groups, adults between 25 and 70 years will be recruited, with half of the sample over the age of 55 years to enable examination of aging effects on HIV x ETOH interactions (Secondary Aim). Participants will be assessed at three time points (baseline, approximately 3-monthsand approximately 9 months), enabling both initial baseline analyses to address Aim 1 and also analysis of the brain and neurocognitive changes following the motivational intervention to reduce ETOH consumption (Aim 2). At each assessment, we will obtain clinical and medical history along with cognitive measures and serum biomarkers. Neuroimaging (fMRI, DTI, T1, MRS and Flair) will be conducted at baseline and 6 months.

1. ***Study Procedures:***

**Informed Consent**

All participants will be fully informed of the purposes and procedures of the study. The informed consent states that participants will be asked to attend up to 4 study visits. Each time point will take between 4 and 8 hours to complete, for a total of no more than 24 hours for the year of participation. These two visits will be repeated at 6 months. The 3 and 12 month visits will consist of the same measures without an additional MRI being conducted. Neurocognitive testing, blood tests, and psychological measures will be collected at these visits. If an individual wishes to participate, he/she will be asked to sign an informed consent form prior to completing the baseline assessment. Participants are provided with a copy of the consent forms, and the originals are kept in a locked file, on site (Florida – Health Professions Nursing and Pharmacy Building)

**Assessments**

There will be four total research study visits (baseline, 3 months, 6 months, and 12 months). Cognitive functioning will be assessed during all time points. MRI brain scans will occur at baseline and 6 month follow-ups. The research study visit at baseline is for the purposes of determining if participants meet study criteria and assessing cognitive functioning; during all visits, study criteria are reviewed to ensure that enrolled participants are still eligible. Prior to determining eligibility, study information is explained by the research assistant and consent is obtained. Additional psychiatric, substance use assessment and neurological questionnaires will be performed. Participants will be asked to bring in recent lab values to verify HIV status, CD4 count, and viral load. Blood, saliva and urine specimens will be obtained and measured of immunological, virologic, and hepatic status and other biomarkers. All blood specimens will be coded**.** Study blood samples will be stored at the McKnight Brain Institute or in CTSI Biorepository located in the Medical Sciences Building, Rooms 621 and 641.De-identified coded samples will be provided to the Biorepository staff for processing and storage. OnCore is the inventory database for the specimens maintained in the CTSI Biorepository. Only authorized users have OnCore access via user name and password authentication. The CTSI Biorepository has a keypad entry, locked freezers, and all visitors have to sign in. Samples and data will only be released to the PI or PI-designated researches with appropriate IRB approval.

A neurocognitive assessment will be performed by a research assistant. The duration will be approximately 4.5 hours.Visits at baseline and 6 months consist of an MRI scan lasting approximately 50 minutes that take place after the research study visit mentioned above. For the participants’ convenience, the research study visit and MRI scan can take place on the same day. Functional imaging will be conducted during this MRI time. All deidentified data will be stored using CTSI resource REDCap. Only personnel listed on the study protocol will have access to database. As this is a multisite study, a multisite REDCap will be created which will allow data to be entered using the same standards and protocol while only allowing data to be seen by approved personnel at their respective site.

**MicroRNA analysis**

5ml of blood and 10ml of saliva will be collected and transferred to Dr. Mavian’s lab in the Emerging Pathogen Institute for downstream analysis. RNA will be extracted using RNeasy Protect Saliva and miRNeasy Serum/Plasma Kits (Quiagen). Participants will be asked not to eat or drink (except water) for two hours prior to saliva collection. They will also be asked to use provided mouth wash 15 minutes prior to saliva collection. *Electron Microscopy:* The concentration, shape, and overall appearance of the exosomes will be examined using a FEI Spirit TEM. *Next-generation sequencing:* Spike-in RNAs (Exiqon) are added to samples and libraries are constructed using TruSeq Small RNA kit and run on a NextSeq 500 platform (Illumina). *Analysis:* After filtering and trimming, reads are aligned to the human genome allowing 1 mismatch, with further mapping miRBase and RFam databases. Single nucleotide polymorphism are identified with miRdSNP database. Reads mapping to the Human Oral Microbiome Database are eliminated. Only uniquely mapped reads for human miRNA are retained for analysis. Reads are aligned to the spike-in controls allowing no mismatches and normalized using the spike-in controls and total number of mapped reads. *Clustering:* MetaMirClust and Regularized Least Squares for MiRNA-Disease Association methods will be employed for unsupervised and semi-supervised clustering, respectively, across samples and per-sample. *Sampling:* Five ml of saliva previously collected will be minimally processed: no exosomal enrichment or RNA isolation will be performed. *Saliva-based microRNA assay:* miRNA of HIV replication and infectivity and disease progression that will be tested are miR-27, miR-28, miR-29, miR-125b, miR-146, miR-150, miR-155, miR-221, miR-223 and miR-3823-4,7-9. Endogenous miR-16 and spiked-in synthetic cel-miR-39 will be used as internal controls. MicroRNA will be purchased from TaqMan MicroRNA Assays (Applied Biosystems). Total RNA (10 ng) is reverse transcribed with Taqman MicroRNA Reverse Transcription Kit (Applied Biosystems) and cDNA pre-amplified with Taqman PreAmp Master Mix (Applied Biosystems). PCR reactions will be performed with ddPCR Supermix (BioRad). Droplet formation and amplification will be carried out with QX200 droplet generator, and analysis with BioRad QX200 reader.

**Intervention**

Heavy drinkers will be randomized by a computer program to either no intervention or Motivational Interviewing (MI) either after the MRI scan or on a separate day. MI will be delivered by master's-level interventionists, located at Brown University, hired, trained, and supervised by Drs. Kahler and Mastroleo located at Brown University (Brown University IRB has approved the MI component via webcam with local study). The interventionists are based at the Center for Alcohol and Addiction Studies at Brown and will conduct the initial session of the intervention, to be completed directly after the baseline visits, and again at 3 months, with participants in this project using a secure HIPAA-compliant videoconferencing system, SecureVideo Video Conferencing, One follow up phone call, also known as the Booster Session, with the Brown interventionist will be completed 1 month after the first MI session. Detailed treatment manuals will be used at all times to ensure standardization of treatment delivery, including a detailed script for the follow up phone session found on page 36 of the Motivational Interviewing Manual. The intervention is the same as the one successfully used in the ongoing clinical trial at Fenway Health (Research Component 2). The primary modification for the current study is that the normative feedback will be tailored for national gender-based norms rather than based on norms for MSM. The intervention can take place after the MRI scan or at a separate appointment.

**Measures**

To confirm participant eligibility, a generalized screening form pertaining to HIV diagnosis, MRI compatibility, and alcohol consumption will be administered along with the Psychosis Screening Questionnaire (PSQ).

A standard medical history/demographic information questionnaire and neurological examination will be administered. All of these measures have already been reviewed and approved by the Brown IRB and are currently in use in Research Component 1.

Structured Clinical Interview for DSM-IV (SCID). Current ETOH and substance use disorders, as well as current major depressive disorder and mania, will be assessed at baseline using the SCID-NP, along with the psychotic disorders screen. Adequate reliability of the Axis I SCID has been demonstrated.

Alcohol Use Disorders Identification Test. Developed by the World Health Organization (WHO) as a simple method of screening for excessive drinking and to assist in brief assessment.

Lifetime Alcohol and Drug Use History. This interview will be conducted at baseline to gather information about age of onset of alcohol use, period of heaviest drinking, most drinks consumed on a single day, and number of drug classes used lifetime. It is based on the questions used for screening for alcohol use disorder for the Composite International Diagnostic Interview, and has been shown to provide discriminating data regarding lifetime alcohol involvement.

Kreek-McHugh-Schluger-Kellogg (KMSK) Scale. Developed as a research measure of drug use severity in accordance with DSM-IV criteria, the KMSK is reliable and valid, quantifying frequency, duration, and amount of individual drug use.

Timeline Followback (TLFB). The TLFB interview will be used to assess recent alcohol use at baseline, as well as during the follow-up intervals. The TLFB interview is a calendar-assisted structured interview which provides a way to cue memory so that accurate recall is enhanced. A structured interview of drinking behavior has been found to be the most reliable and valid method of assessing prior alcohol use. The TLFB interview has excellent reliability and validity. At baseline, it will be administered for the 1 month prior to the interview (to reduce subject burden and assessment reactivity) and will provide data on the percentage of drinking days, drinks consumed per week, and the percentage of heavy drinking days. The TLFB can be modified readily to provide a valid assessment of drug use behavior. At baseline, the TLFB will provide data on the number of drug classes subjects used and the frequency of drug use. The TLFB will be used to determine criteria for heavy and moderate drinking.

Urine Drug Screen. In addition to providing a breath sample for alcohol concentration analysis at each session, participants also will provide a urine sample for testing for recent drug use. Urine drug screens will be performed using the Drugsmart Cup to test for benzodiazepines, amphetamines, THC, opiates, & cocaine.

The Short Inventory of Problems [SIP] will be used to assess the extent to which subjects have had problems related to their alcohol use. The SIP assesses 15 negative consequences of alcohol use over the chosen time period (in this case 3 months) and has been found to have good psychometric properties.

The Epworth Sleepiness Scale [ESS] will be used to assess the participant’s level of general alertness and likelihood of falling asleep. The ESS proposes eight daytime scenarios, and the participant is asked to rate how likely they are to fall asleep in each situation on a four-point scale (0-3).

Self-report inventories of psychopathology and behavioral problems will be given at each evaluation to assess for 1) psychopathology, 2) depression severity, 3) apathy, 4) fatigue, 5) impulsivity, and 6) quality of life. The Center for Epidemiological Studies-Depression Scale (CES-D) to assess depression severity has been used in previous HIV studies (e.g., our HERS studies), with adequate norms and good reliability and validity. We will also administer the Beck Anxiety Inventory and the Perceived Stress Scale. We will also administer the Early Life Stress Questionnaire (ELSQ) to assess stressful experiences and situations encountered in childhood and adolescence. The Frontal System Behavioral Scale (FrSBe) will assess behavioral change, including apathy, impulsivity, and executive dysfunction associated with brain disorders. The BARRATT Implusivity Scale is a global measure of the personality/behavioral construct of impusliveness. The Medical Outcomes Study HIV Health Survey (MOS-HIV)

based on the SF-36 will be given at each assessment. It provides physical and mental health composite indices to measures of self-perceived health status and QOL. Nine subscales assess functional impact on physical and mental health. In addition, Medication Adherence, Sexual Behavior Questionnaire, Alcohol Use Contemplation, Alcohol and ART Interaction questionnaires will be given for the purpose of preparing report for MI session.

A standard battery of neurocognitive tests will be administered at baseline, 3 months, 6 months and 12 months. These tests are well-established the field of HIV-associated neurocognitive dysfunction and have been used extensively by our team in previous studies (e.g. Age Effects). The following neurocognitive tests will be administered:

1. MoCA
2. WTAR
3. HVLT-R
4. WAIS-3 Letter Number Sequencing
5. WAIS-3 Digit Symbol
6. WAIS-3 Symbol Search
7. Trail Making
8. Grooved Pegboard
9. Stroop Task
10. COWAT
11. Animal Fluency
12. BVMT-R
13. PASAT-1
14. WAIS-3 Vocabulary
15. Boston Naming Test
16. ARCPT
17. CalCAP
18. N-Back
19. Set switching paradigm

MRI exams will be conducted at the University of Florida AMRIS facility. The procedure takes about 50 minutes to complete. During the scanning session, brain MRI and MRS data will be obtained using a series of sequences in a pre-constructed imaging protocol: 1) T1 MPRAGE; 2) FLAIR; 3) DTI; 4) MRS 5) fMRI. During approximately 15 minutes of the scanning time, the set-switching paradigm and N-Back Task will be administered. Any abnormal incidental findings will be reported to the study PI for review. If deemed appropriate, the MRI images will be sent to a staff radiologist for further review. An investigator will inform the participant that an abnormality has been found and advise the participant to contact their primary care doctor.

In the event that a participant is unwilling or unable to attend a follow-up visit in person, they will be asked to participate in a brief phone call assessing their substance use since their previous visit.

Compensation

Participants will receive $75 for completion of thinking and memory tasks at baseline and 6 months, and they will receive $125 for completion of each MRI scan visit, including the baseline MRI and 6 month MRI. Participants will also receive a $75 gift card for completion of thinking and memory tasks at 3 month and 12 month follow-ups for a total compensation of up to $550. A $10 gift card will be given to participants who travel 20-39 miles one way from study site, and an additional $10 gift card will be given to participants who travel >40 miles one way. A $15 gift card will be given to participants who do not have reliable transportation and are required to use public transportation in order to attend study visits. We may also offer to arrange and pay for round trip transportation to and from study visits. In the event a participant is excluded during the research study visit they will be given a $10 gift card to thank them for their limited participation. If participants attempt but are unable to complete the imaging, they will receive full compensation for that visit, but will be withdrawn from the study.

1. ***Safety monitoring.***  In an effort to meet the NIH policy for Data and Safety Monitoring, we have created a system for oversight of the project. Oversight of internal monitoring of the participants’ safety will be conducted by the project and Florida site PI, Dr. Cohen. Investigators on this application have extensive experience with clinical and observational studies with HIV-infected patients. All participants will be screened for MRI safety prior to undergoing MR imaging, or entering the MR suite. The subject safety screening documents utilized at the University of Florida Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility and UFHealth/Shands are attached as Appendix A to this proposal, and will be used as a screening tool for the study.

Entities Conducting Monitoring

The Institutional Review Boards (IRBs) the University of Florida and Brown University will review this protocol and all procedures and will provide oversight. The site Principal Investigators, Dr. Karen Tashima and Dr. Cohen, the University of Florida IRB and the Brown University IRB will conduct monitoring.

What is monitored?

Monitoring is done of all procedures to ensure that they conform to the approved protocol; of unforeseen circumstances that might arise and affect safety; of all reports of serious adverse events as defined in 38 CFR 46 and the FDA 312.32 (death, life-threatening experience, new or prolonged hospitalization, persistent or significant disability/incapacity); of other significant adverse events (adverse events that lead to drop out by participant or termination by the investigator); of unexpected adverse events resulting from the study; social harms reported by participants; and of expected adverse events.

Monitoring is done of all study inclusion and exclusion criteria. During this clinical trial, we will notify officials, as mandated by law, if a participant reports intentions to harm him/herself or others, or reports child abuse or abuse of an elder. The project and Florida site PI, (Dr. Cohen) is a licensed psychologist, who will be available on call in case of any psychological adverse events, occurring at the Florida site. In the event a participant were to report a need or interest in treatment for alcohol/substance dependence, psychiatric disorder, or distress, an appropriate referral will be provided based on an extensive list of referral resources maintained by the study.

Frequency of Monitoring

All adverse events will be continuously monitored by the PI. Participants will be given contact information so that they can inform us of events that occur in between study visits. Drs. Cohen will conduct daily oversight of participant safety. He will meet bi-weekly with staff to review participant progress and their experiences with the experimental procedures, including adverse events. Any adverse events that are observed and/or reported will be immediately reported. The Investigators will be available to meet outside of the weekly meetings, if necessary, due to concerns regarding a particular participant or any problems that may arise for participants. If necessary, they will make appropriate recommendations for changes in protocol.

The University of Florida, and Brown University conduct the monitoring at the continuing reviews as scheduled, whenever modification requests are considered, and upon receiving reports of serious adverse events from the PI or anyone else. NIAAA monitors the study upon receipt of annual progress reports and whenever other information is received.

Reporting Plan:

Any serious adverse events that are observed and/or reported will be immediately reported to Dr. Cohen (Florida site). Serious adverse events are then reported to the University of Florida IRBs and to NIH. All other adverse events related to the study will be reported at the continuing review. Serious adverse events will also be reported in writing to the NIAAA Project Officer within 48 hours. All serious adverse events related to the study will be reported annually in the Progress Report sent to the NIAAA Project Officer.

Any actions taken by the IRB, other than acceptance of the adverse event report, will be reported to NIAAA along with any changes or amendments to the protocol requested by the IRB in response to these reports. NIAAA will subsequently be informed of any substantive changes or amendments in approved protocol.

Confidentiality

Confidentiality is maintained as follows. Data files recorded with an identification number are stored in locked offices in the Health Professions, Nursing and Pharmacy building(Florida site) and are accessed only by project staff. All project staff are knowledgeable about confidentiality and human subjects protection. Follow-up contact forms that require identifying information is stored separately from data files and are accessed only by those staff conducting follow-up interviews. All specimens are coded by subject ID number only before being sent to the Virology Core for testing. Data files contain no identifying information, including date of birth and town of residence. Data are transmitted to the Data Core in encrypted format.

Brown staff conducting videoconferences with participants will do so in room that is private from other Brown staff. During follow-up telephone calls to the participants' homes, no information is provided to others in the household.. All blood samples are coded by a subject ID number. Data files contain no identifying information, including date of birth and town of residence

Data and Safety Monitoring Plan:

Because this is an exploratory study with minimal risk associated with the proposed intervention, there will not be an external data safety and monitoring board for this study. The research team, however, will follow the procedures for data safety and monitoring as required by the Institutional Review Board at the University of Florida (UF IRB). The research team will monitor participants for any potential adverse events, and all reported events will be forwarded by the PI to the UF IRB.

A data and safety monitoring plan (DSMP) has been implemented to ensure the safety of all participants involved in the study and to ensure the validity and integrity of the data. The primary goal of the DSMP will be to monitor the progress of the study and safety of participants and if necessary, recommend modifying the study or terminating the study as appropriate.

* The PI’s will be responsible for coordinating activities of the DSMP, including:
  + Lab Meetings twice per month, to discuss participant involvement in the study, and any issues that have developed or need revision
  + Safety concerns
  + Data Storage and safety
  + Participant safety
  + Outcome data
  + Data quality
  + Integrity
  + Intervention efficacy
  + Recruitment
  + Performance

Both the PI and the study staff will review and examine reports of adverse incidents immediately, and make reports to the IRB as necessary for any and all of the following situations:

* Serious and non-serious adverse events that may occur
* Suspicion of scientific fraud or misconduct
* Any other issues which may warrant protocol changes or modifications.

**Procedure for collection and storage of data.** A number of quality control procedures will be used to ensure the validity and integrity of the data and the safety of all participants involved in the study. Relevant data and safety information obtained on each study participant will be verified against the original source documents by the primary study coordinator and any identified discrepancies will be reviewed at these meetings. The primary goal of these meetings will be to monitor the progress of the study and safety of participants and if necessary, recommend modifying the study or terminating the study as appropriate.

All identifying information will be archived in Dr. Cohen’s neuroimaging laboratory within the Center for Cognitive Aging and Memory at UF*.* Imaging data will undergo several levels of processing, and all raw and processed data will be archived on a University password-protected server in password-protected folders and files. Only study staff will have access to these files. The self-report data will be double entered using the RedCap Data System. This system signals the user when an out-of-range value is entered. All data entry is then verified via double-entry, with the program signaling mismatches with the original entry. Next, computer-generated reports of variable frequencies and subject lists will be reviewed, leading to possible corrections to coding or entry. After checking for accuracy of data within a given group, data will be stored in the password-protected folders along with the imaging data.

Location and logistics of data collection. Procedures involving human subjects will be performed at facilities of the UF Health Care System. Neurocognitive testing will be performed at the Clinical and Translational Research Building (CTRB). Several clinical research examination rooms are equipped and dedicated to neurocognitive and functional assessment, and contain all necessary computers and test materials. Storage of neuroimaging data backup will take place on the University of Florida’s Research Computer HiPerGator system. Neuroimaging will take place at the AMRIS facility of the McKnight Brain Institute, which is located two blocks from the CTRB. For the Motivational Interviewing intervention participants will be located at the CTRB and will engage via secure teleconferencing with interventionists at Brown University MI data and recordings will be stored securely in Brown IRB approved locations.

Storage of collected data. All electronic data are stored in password protected, secured computer systems. All paper data will be stored in a locked file cabinet. Data will only be removed when coded, entered, or audited. Only the participant’s study identification number will appear on any data forms. Only the PI, the Co-Is, and the RAs will have access to the completed data forms and electronically stored data. All data are considered part of the participant’s confidential record. Data collected from research participants will be stored in a secured, password protected computer file that is separate from network systems. All paper data (e.g., subject contact information, consent forms, etc.) will be placed in a locked file cabinet within 24 hours of their acquisition as designated by the study's RA . All data will remain confidential.

Data entry requirements. The data entry system will require a login identification and password in order to gain access to the data. Where appropriate, validation and range rules will be applied to the actual entry fields. Only study staff will be able to view the data in its raw state.

Audit/verification of entered data. All data designated as primary outcome data will be subject to a 100% cross-referencing between electronic and paper forms. This audit must have an error rate less than 1%. If the verification fails the audit, all data will be re-entered, the original computer files discarded, and the newly re-entered data audited. This process will continue until the audit no longer exceeds the maximum allowable error rate. All audits will be supervised and documented by the PI.

Data management and analysis. Our research team has substantial experience in the design and implementation of data management procedures that provide accurate recording and storage of data, participant confidentiality, and timely analysis. Based on our past experience, we believe that our major data management and analysis needs for the proposed project can be met by using a high-end PC, equipped with the latest version of SPSS for Windows and appropriate spreadsheet programs. All data files are automatically backed-up daily.

Data quality control. All staff involved in data collection will be trained and certified to ensure their competence, and re-certified periodically throughout the study as we have done in similar trials. Data will be collected and numerically coded using pre-tested electronic entry forms. At the time of collection, there will be initial clerical review of all data for accuracy and completeness. Every effort will be made to ensure that missing data are kept to a minimum. Data entry programs with range checking and response validation will be used for all data entered. Under supervision from the PI, the data manager will conduct error checking procedures and preliminary analyses on all data to ensure their accuracy. The RAs will be trained to avoid omissions in data entry and computer entry protocols will be programmed to avoid accidental skipping of question items. We believe that the quality control system to be used will ensure a complete and accurate database, and maximize the likelihood that the intervention will be delivered correctly and efficiently. As we have done in prior studies, a manual of procedures will be developed during the initial study start-up period that explicitly describes the specific procedures related to intervention delivery, data collection, and quality assurance.

Frequency of data review. Relevant data and safety information obtained on each study participant will be verified against the original source documents by the primary study coordinator on a bi-weekly and reviewed at weekly meetings.

Measurement and reporting of participant accrual and adherence to eligibility criteria. Review of the rate of participant accrual, adherence to inclusion/exclusion criteria will occur weekly during the recruitment phase and then every month to assure that participants meet eligibility criteria and ethnic diversity goals outlined in the grant proposal.

1. **Possible Discomforts and Risks**

Potential risks in the study are considered moderate and include 1) potential discomfort related to completing questionnaires about sensitive information such as alcohol/drug problems and completing cognitive assessments; 2) potential discomfort during blood draws for viral load, CD4, and liver function tests; 3) potential breach of confidentiality and/or privacy; 4) Symptoms of alcohol withdrawal

Risks of MRI Assessment. The MRI may involve some slight discomfort. The MRI involves lying on a table in an enclosed space. The small space of the MRI is generally not a major source of discomfort, though it may produce mild emotional discomfort among some people who fear small spaces. Although MRI is normally a safe procedure, there have been rare incidents where patients have received skin burns. Even more rarely, the high magnetic field has dislodged or interfered with the operation of metallic implants or devices; i.e. the magnet associated with the MRI can be hazardous to individuals with metal in their body. In a few of these cases, the injury has been fatal. The magnet will create loud noises. Most people find the sound quite tolerable, butit have been reported to produce temporary hearing loss in a very small number of people. Participants will be provided with earplugs to wear in the scanner, and also headphones to reduce this risk and possible discomfort.

**Protection Against Risk**

Potential risks of the study are considered moderate. For the possibility of subjective discomfort from answering questions or from cognitive assessments, any distress will be minimized by assurance that participants can refuse to answer any question that they do not feel comfortable addressing and may withdraw at any time without penalty. Interviewers are skilled in talking about sensitive information with subjects, and subjects may decide to end an interview at any time. Subjects will be asked to provide urine and blood samples for testing. Potential discomfort during the blood draw and urine collection will be explained during informed consent. Subjects can choose to withdraw from the study without penalty. Breach of confidentiality is highly unlikely because all information will be identified with a code only and stored in a locked file cabinet. An enrollment database linking names and study identification numbers will be kept in a secure folder separate from other subject data sources and will be used to facilitate the collection of follow-up data. Only grant staff will have access to this database. All staff are or will be fully trained in relevant ethical principles and procedures, including confidentiality. All assessment and treatment procedures will be closely supervised by the site PIs. All audiotapes will be erased upon completion of data analysis.

MRI Risks. Participants will be provided earplugs and/or headphones to reduce the noise. The risk of discomfort due to small spaces will be explained to participants prior to entering the scanner. A screening will be conducted by medical personnel prior to the MRI. Individuals with metal in their body will not undergo the MRI. Pregnant women will not be scanned.

During this study, we will notify officials, as mandated by law, if a participant reports intentions to harm him/herself or others, or reports child abuse or abuse of an elder. Our rigorous screening procedures will help to ensure that consenting volunteers are recruited who do not report significant psychiatric disturbance. However, in the event a participant were to report a need or interest in treatment for substance dependence, psychiatric disorder, or distress, an appropriate referral to resources will be provided.

1. **Possible Benefits:**

The proposed research may provide several potential benefits to subjects and others. Subjects will be participating in a study that may provide knowledge to guide the future treatment of HIV-infected patients who drink heavily. Given that the risks to subjects are considered to be moderate, the risk-benefit ratio is deemed favorable. In addition, this study has the potential to contribute valuable information about the potential for brief alcohol interventions to reduce drinking in patients in HIV primary care and about the effects of reducing drinking on a range of important HIV-related biomedical and behavioral outcomes. Developing effective interventions that address alcohol use in HIV patients can make a major public health impact by reducing the morbidity, mortality, and transmission risk associated with heavy alcohol use. Given the substantial health risks associated with heavy drinking in HIV patients, the importance of the potential knowledge to be gained relative to the subject risk is favorable.

1. **Conflicts of Interest:**

There are no conflicts of interest.