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Summary results for ClinicalTrials.gov

Title: NURA-007-17F, HCV and co-morbid alcohol use disorder: a translational investigation of antiviral therapy outcomes on CNS function

NCT number: NCT03902366

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Thank you for the opportunity to conduct this research study.

Please contact me with any questions.

Sincerely,

Jennifer M. Loftis, Ph.D.
Principal investigator

Research Protocol

Title

HCV and Co-morbid Alcohol Use Disorders: A Translational Investigation of Antiviral Therapy Outcomes on CNS Function

Investigators

Principal Investigator: Jennifer Loftis, Ph.D.
Responsible Clinician: Michael Chang, M.D.

Specific Aims/Purpose

The primary objective of this research project is to compare neuropsychiatric functioning, cortical activity, white matter integrity, and immune response among Veterans with and without alcohol use disorder (AUD), before and after direct-acting antiviral (DAA) therapy [a new treatment for chronic infection with the hepatitis C virus (HCV)]. Demographically-matched comparison groups of Veterans without HCV (HCV-, with and without AUD) will similarly be evaluated to determine the relative contribution of HCV and an HCV "cure" to outcomes putatively affected by alcohol abuse.

Two specific aims are proposed.

Aim 1: Determine the impact of DAA therapy and a sustained viral response on central nervous system (CNS) function
Aim 2: Evaluate the effects of AUD and unhealthy alcohol drinking on DAA therapy outcomes and CNS function.

The information learned will address a critical gap in our knowledge concerning the effects of alcohol use on DAA therapy outcomes and will help inform treatment guidelines that could be translated to clinical practice, such as targeted interventions to treat AUD in conjunction with HCV infection and follow-up strategies for patients who successfully complete DAA therapy but then need care for other potential CNS-related outcomes.

Central hypothesis: It is hypothesized that adults with HCV and co-morbid AUDs may be at increased risk of persistent brain dysfunction following DAA therapy.

Specific hypotheses: *Hypotheses 1.1 and 2.1 (neuropsychiatric outcomes)*. Following DAA therapy and obtaining a sustained viral response (SVR), 1.1: participants will show improved neuropsychiatric outcomes (*e.g.*, cognitive function, fatigue, mood), as compared to baseline (pre-DAA therapy). 2.1: participants without AUDs will show greater improvement in neuropsychiatric outcomes than participants with AUDs. *Hypothesis 1.2 and 2.2 (neuroimaging outcomes)*. Reduced fractional anisotropy (FA) will be observed in the tracts that connect regions between which there is decreased functional connectivity. 1.2 and 2.2: Disintegrity within white matter tracks [as measured by FA and mean diffusivity (MD)] observed at baseline visits will be improved following DAA therapy, with greater improvement observed in participants without AUDs. On the fMRI Balloon analogue risk task (BART) and Monetary incentive delay task (MID), adults with HCV will be significantly more likely to display more impulsivity during the baseline visit, as compared to the post-DAA therapy visit. It is hypothesized that AUD may attenuate the positive antiviral therapy effects on impulsivity. *Hypothesis 1.3 and 2.3 (immune activation profile)*. Inflammatory profiles will be associated with neuropsychiatric impairments and alterations in white matter integrity and cortical activity. Following DAA therapy and obtaining an SVR, 1.3: participants will show reduced immune activation profiles (*i.e.*, restored T cell phenotypes and a reduced inflammatory profile), as compared to baseline, and 2.3: participants without AUDs will show greater a reduction in inflammatory factors than participants with AUDs.

Scientific Rationale and Significance

Direct-acting antiviral (DAA) therapies are being widely used to treat patients with chronic HCV infection. The Department of Veterans Affairs (VA) is at the forefront of these transformative treatment efforts and is now offering DAA therapy to all Veterans with HCV who are treated within the VA health care system (Graham, 2016), with estimated viral clearance rates of ~90% (Fitz, 2016). Despite this progress, there are limited data on long term physical health outcomes following DAA therapy, and there are no published studies on alcohol addiction and mental health outcomes—outcomes that affect daily life such as cognitive function and substance abuse behavior. Given that alcohol use disorders (AUDs) are almost three times more prevalent in Veterans with HCV compared to those without HCV infection (Butt et al., 2007), and similarly, that adults with HCV report greater alcohol intake than other adults (Taylor et al., 2016), longitudinal studies are needed. Chronic HCV infection is often accompanied by impairments in cognitive function and mood, but whether these adverse effects are caused by HCV or by the co-morbid substance abuse has been challenging to disentangle. Unlike interferon-alpha (IFN-α) based therapies (previously the standard of care for HCV treatment within VA health care systems), DAA therapies are not associated with neuropsychiatric side effects or the induction of inflammatory signaling cascades—making it possible for the first time to investigate interactions between alcohol use and viral clearance on CNS function and immune response. It was recently reported that; "…we do not have enough data on mental health after HCV eradication with the newer antiviral agents…the currently available data call for a more intense study of the neuroimmunological and neuroinflammatory consequences of HCV" [(Weissenborn and Tillmann, 2016); see also (Solinas et al., 2015)].

Relevance to Veterans' health: The introduction of DAA therapies are expected to significantly improve viral clearance rates and treatment outcomes. The goal for the VA is "to eradicate as much of the disease as we can," said Chester Good, M.D., Chair of the VA's medical advisory panel for pharmacy benefits management (JAMA, Aug. 10th, 2016). Yet, there are very few data on DAA therapy outcomes. The titer of HCV is not always predictive of the clinical course. It has been argued that SVRs may not be adequate to assess HCV treatment outcomes (Gurusamy et al., 2013), and unlike the HIV titer, little effort has been made to validate the SVR. This research study will generate CNS-focused outcome data following DAA therapy that can be used not only to help guide treatment strategies for HCV and co-morbid AUD, but also may contribute towards validating the SVR as a surrogate marker of HCV cure. A recent retrospective study among adults treated at VA health care systems found that the majority of patients with HCV who drink unhealthy amounts of alcohol can obtain an SVR following DAA therapy (Tsui et al., 2016); however, it is important to recognize that continued alcohol use, even after HCV eradication, may hinder improvements in mental health and cognitive function and put patients at risk for progression of liver disease. Despite high likelihood of cure for most patients with HCV in the DAA era, rates of HCV are increasing over time (CDC, 2017), and unhealthy alcohol use influences treatment completion and outcomes (Anand et al., 2006, Grodensky et al., 2012). Thus, studies to inform evidence-based decision-making for patients with co-morbid AUD and HCV are needed [*e.g.*, (Boyd et al., 2012)]. Among VA patients being treated for HCV, 44% have a documented alcohol use disorder (Tsui et al., 2016), and unhealthy alcohol use is expected to be disproportionately represented among those who are awaiting treatment, given previous contraindications to HCV treatment in this population.

Preliminary Studies

To investigate how HCV and co-morbid AUD contribute to neuropsychiatric impairments, altered immune responses, and CNS abnormalities, the following work has been accomplished.

STUDY 1. This study of 55 male Veterans with HCV was conducted at VA Portland and Long Beach Health Care Systems and results were presented at the Digestive Diseases Week conference (McCready et al., 2017) and recently accepted for publication in *Alcohol Clinical & Experimental Research* (Loftis et al., in press). Adults with HCV (not undergoing treatment for HCV) and co-morbid AUD [n = 42; mean age (SD) = 59 (5.55)] who were participating in a brief psychosocial treatment intervention to change alcohol use behavior were compared to adults without AUD [n = 13; mean age (SD) = 60 (4.62)]. Participants were evaluated at three time points to assess changes in alcohol use and inflammatory factors over a 12-week period. The Beck Depression Inventory, 2nd Edition (BDI-II), Posttraumatic Stress Disorder Checklist (PCL-C) (Karstoft et al., 2014), AUD Identification Test-Consumption (AUDIT-C), and Timeline Followback (TLFB) were used to assess psychiatric function and alcohol use. Indices of immune activation, blood brain

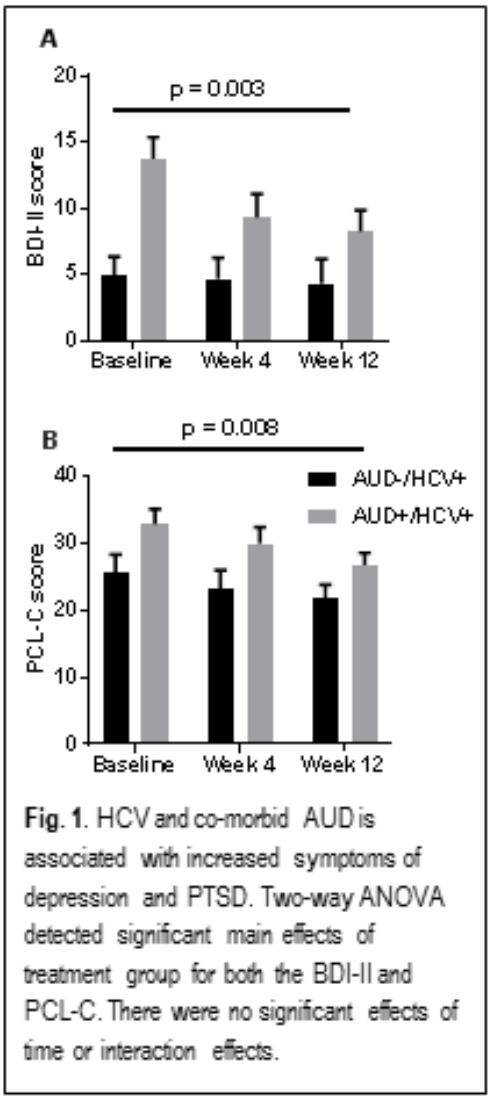

Fig. 1. HCV and co-morbid AUD is associated with increased symptoms of depression and PTSD. Two-way ANOVA detected significant main effects of treatment group for both the BDI-II and PCL-C. There were no significant effects of time or interaction effects.

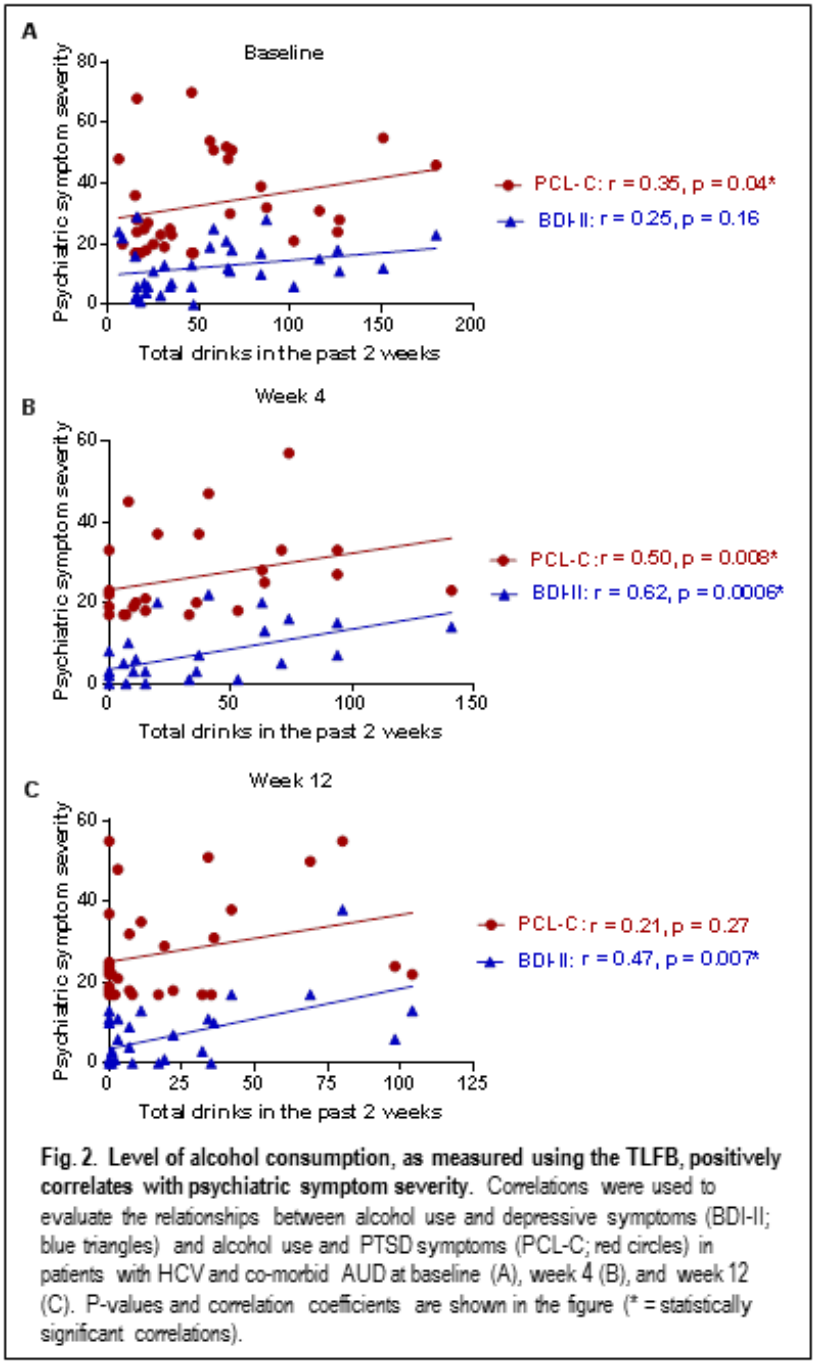

Fig. 2. Level of alcohol consumption, as measured using the TLFB, positively correlates with psychiatric symptom severity. Correlations were used to evaluate the relationships between alcohol use and depressive symptoms (BDI-II; blue triangles) and alcohol use and PTSD symptoms (PCL-C; red circles) in patients with HCV and co-morbid AUD at baseline (A), week 4 (B), and week 12 (C). P-values and correlation coefficients are shown in the figure (* = statistically significant correlations).

barrier damage (S100B), liver function, and viral load were measured in blood using immunoassays and PCR assays.

Neuropsychiatric function. Co-morbid AUD was associated with increased symptoms of depression and PTSD (**Fig. 1**). Among participants in the AUD group, there was a significant reduction from baseline to 12 weeks in number of alcohol drinks per two-week segment ($p < 0.0001$), and nine participants in the AUD reported obtaining abstinence from alcohol by week 4. Alcohol use (as measured by the TLFB) was positively correlated with the severity of depressive and PTSD symptoms (**Fig. 2**).

Immune activation biomarkers. Blood samples from the same study of participants with HCV (with or without co-morbid AUD) were analyzed for the expression of immune factors and a subset of results are shown in **Figures 3** and **4**. For the data presented in **Figure 3**, plasma samples were analyzed with a multiplex bead-based immunoassay conducted to measure peripheral immune biomarkers associated with inflammation, mood disorders, and infection control [*i.e.*, C-reactive protein (CRP), IL-1β, IL-1 receptor antagonist (IL-1RA), IL-6, IL-8, IL-10, monocyte chemoattractant protein-1, tumor necrosis factor-α (TNF-α)]. Results from three of the factors are shown (CRP, IL-10, and IL-1RA). **Figure 4** summarizes data on S100 calcium-binding protein B (S100B) levels—a common marker for BBB permeation, which is expressed primarily by astrocytes that cover blood vessels. To investigate the effects of AUD on BBB integrity in Veterans with HCV, we measured S100B in plasma samples using enzyme-linked immunosorbent assays using previously published methods (Loftis et al., 2013). Taken together, results from STUDY 1 indicate that HCV and co-morbid AUD is associated with increased symptoms of depression and anxiety and altered levels of immune factors [*e.g.*, IL-10, S100B—biomarkers that accompany neuropsychiatric impairments and brain pathology (Chen et al., 2017, Huckans et al., 2014)].

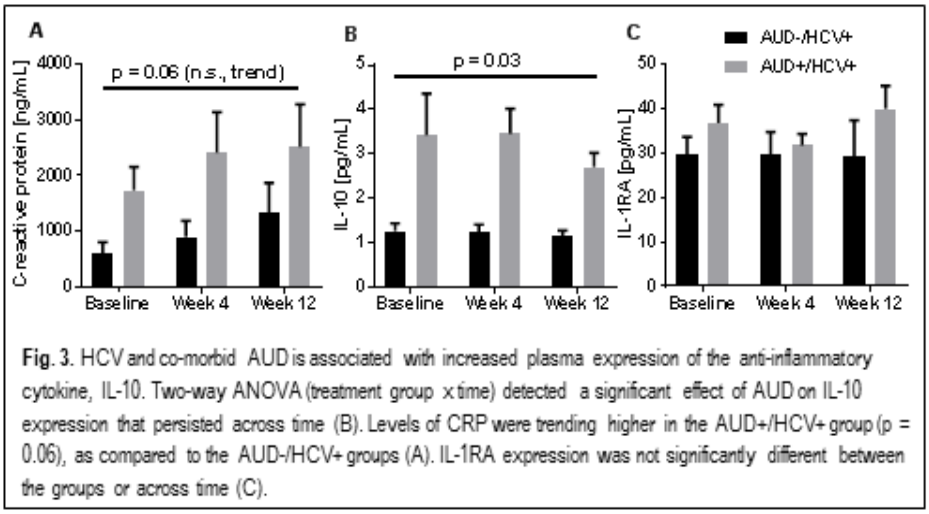

Fig. 3. HCV and co-morbid AUD is associated with increased plasma expression of the anti-inflammatory cytokine, IL-10. Two-way ANOVA (treatment group x time) detected a significant effect of AUD on IL-10 expression that persisted across time (B). Levels of CRP were trending higher in the AUD+/HCV+ group ($p = 0.06$), as compared to the AUD-/HCV+ groups (A). IL-1RA expression was not significantly different between the groups or across time (C).

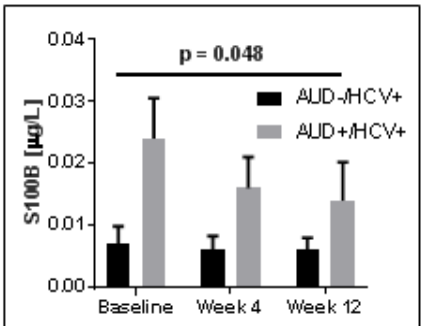

Fig. 4. Veterans with HCV and co-morbid AUDs have elevated plasma S100B levels. Two-way ANOVA indicated a main effect of treatment group ($p = 0.048$). There was no significant effect of time or interaction effect.

STUDY 2. Our group previously reported that adults with HCV (with and without a history of substance abuse) show greater discounting of delayed rewards (a measure of impulsivity that correlates with executive function) than adults without HCV (Huckans et al., 2011). In a follow-up study, we hypothesized that levels of impulsivity would be associated with differences in brain activation when choosing between easy and difficult reward choices.

Functional MRI and delay discounting. Age-matched adults with (n = 22) and without (n = 24) HCV completed three runs of delay discounting (DD) task while undergoing functional T2-weighted echoplanar imaging on a 3T Siemens Magnetom Trio. In preliminary analyses, across participants, the hard vs. easy decision contrast was associated with activation in the medial prefrontal cortex (mPFC). There were no statistically significant group differences in any of the contrasts. However,

examination of the relationship between area above the curve (AAC) (as a measure of impulsivity) and brain activation in the hard vs. easy contrast revealed a significant group by AAC interaction on activation in bilateral dorsolateral PFC, inferior frontal gyrus, insula, and superior parietal lobule. Post hoc analyses detected a positive relationship in controls but a negative relationship in the HCV group. In the HCV group, AAC was negatively correlated with left insula activation. Given the insula's roles in the processing of reward or anxiety and in regulating impulsive behavior, the negative correlation observed suggests that impulsive behavior in patients with HCV may be associated with altered insula function (**Fig. 5**). In addition, for the HCV group, duration of HCV infection was positively correlated with activation in the mPFC (r= 0.458 and p= 0.021). These and related preliminary findings from this study were presented at the Organization for Human Brain Mapping conference (McCready et al., 2017*)* and a full manuscript is under review for publication in the *Journal of Neurovirology*.

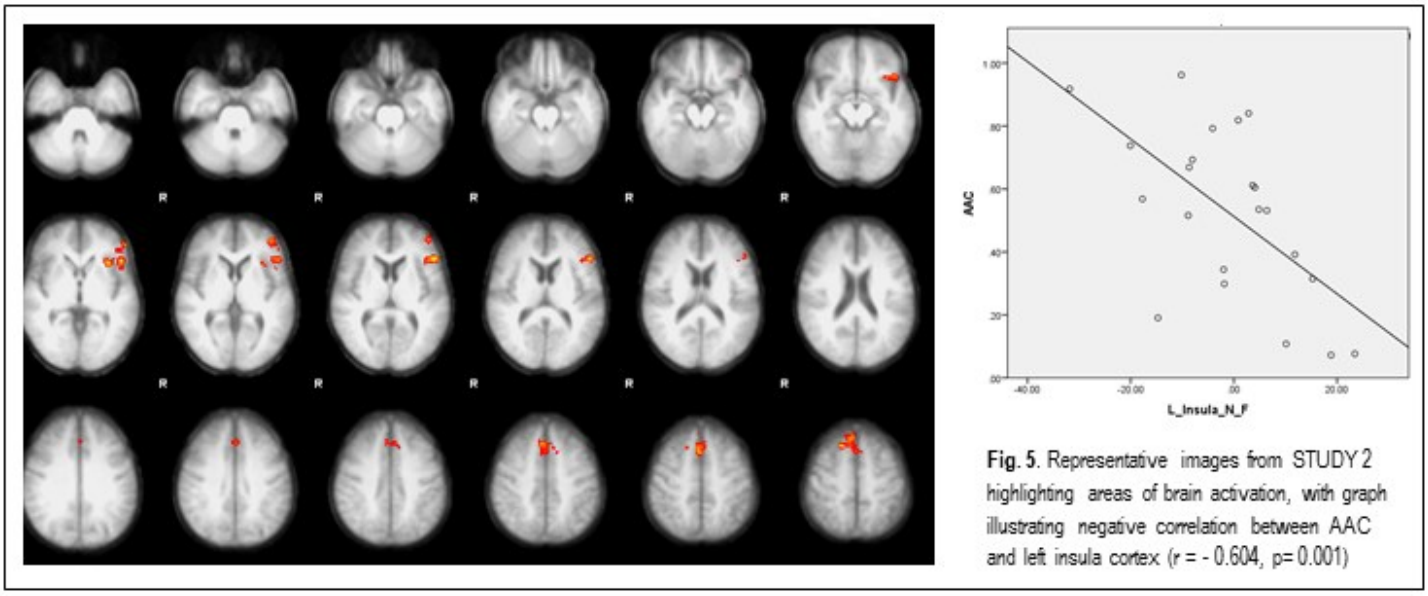

Fig. 5. Representative images from STUDY 2 highlighting areas of brain activation, with graph illustrating negative correlation between AAC and left insula cortex (r = - 0.604, p= 0.001)

Summary of work accomplished. Taken together, the work accomplished is beginning to suggest immunological mechanisms and identify key brain circuits where co-morbid HCV infection and AUD exert adverse effects on brain and neuropsychiatric function. *What remains to be determined is how DAA therapy-induced HCV clearance impacts these CNS functions*.

In published work, we reported post-hoc exploratory analyses that were conducted to determine whether SVR contributed to cognitive and psychiatric outcomes in adults with HCV who had undergone IFN-based therapy. Patients with SVR following IFN therapy (n = 19) had significantly less fatigue and reported fewer symptoms of depression, as compared to patients without SVR (n = 6). No significant relationship between SVR and cognitive function was found; however, IFN therapy was not associated with significant changes in cognitive performance in our sample of patients [(Huckans et al., 2015), Table 5 in Huckans et al., 2015 provides a summary of selected published studies prospectively following the neuropsychological performance of adults with HCV across IFN therapy]. DAA therapies, unlike IFN-based therapies, are not associated with neuropsychiatric side effects or the induction of inflammatory signaling cascades. Further, active AUDs are not considered contraindications to DAA therapy, as they were with IFN-α therapies. *Thus, it possible for the first time to investigate interactions between alcohol use and viral clearance on CNS function and immune response—outcomes that will be critical to assess following DAA therapy and HCV clearance.*

Research Design and Methods

Overview. We plan to conduct a longitudinal cohort study and evaluate participants with (n = 30) and without (n = 30) active AUD at two time points [baseline (prior to DAA therapy initiation) and 12 weeks post-therapy (SVR assessment time point; SVR = negative for HCV RNA 12 weeks after the end of DAA therapy)]. **Table 1** provides a timeline for participant enrollment and study completion. Comparison groups of adults with: i) active AUD and no HCV (n = 30) and ii) no AUD or HCV (n = 30) will also be studied. Thus, we will enroll 120 participants into the following four study groups: 1) AUD+/HCV+, 2) AUD-/HCV+, 3) AUD+/HCV- and 4) AUD-/HCV-. Evaluations will incorporate neuropsychiatric assessments, brain imaging [resting state (rs) magnetic rsMRI, functional (f) MRI (fMRI), and diffusion tensor imaging], and laboratory methods [*e.g*., multicolor flow cytometry, quantitative polymerase chain reaction (PCR), and multiplex immunoassays] to assess the interactive effects of alcohol use on brain and immune function [*e.g*. changes in T cell response and inflammatory cytokines, such as TNF-α] that contribute to viral persistence and neuropsychiatric impairments. We will use general linear models to assess whether average change in the response (*e.g*., neuropsychiatric and immunologic outcomes) over six months between post- and pre-DAA therapy for each subject differs among the four groups—either due to AUD, HCV, or the potential interaction of these factors—after controlling for other subject-level covariates that remain constant (or nearly so) over the 6 months (*e.g*., age, sex). Variables that could meaningfully vary over time (*e.g*., TLFB score) will be summarized in terms of a difference and then included as a covariate.

Table 1. Overview of human subjects procedures, including research visits and HCV treatment schedule[a] (as per standard of care)

Weeks					
-2	**0**	**12**	**18**	**24**	**26**
Baseline visits (wks -2-0)[c] - Clinical interview - Neuropsychiatric assessment - Specimen collection - Neuroimaging evaluation	DAA therapy initiation (wk 0)	DAA therapy completion (wk 12) ETR assessment (wk 12) - Check-in w/participants[b]	- Check-in w/participants[b]	SVR assessment (wk 24; 12-wk post DAA therapy) **6-month follow-up visits (wks 24-26)**[c] - Neuropsychiatric assessment - Specimen collection - Neuroimaging evaluation	

[a]In addition to the visits outlined in this table, Veterans receiving DAA therapy participate in two standard, follow-up clinic appointments at weeks 4 and 8 (typically 15-20 min appointments). *Note: The follow-up clinic is a large multidisciplinary clinic comprised of doctors, fellows, nurse practitioners, liver clinic pharmacist and pharmacy students. Patients do not usually see the same clinician at the follow-up appointments that they saw at the start appointment. In addition, approximately 50% of individuals started on DAA therapy are followed in the pharmacist phone clinic, and the week 4 and 8 appointments are by phone (~10 min calls).* The potential impact of these additional interactions on study outcomes is discussed in Expected outcomes and alterative considerations. [b]Clinical research staff will check-in with participants (phone contact) to assess general well-being, complete the TLFB (Sobell et al., 1996), and confirm schedules for follow-up visits. [c]Participants will be paid $125 for the baseline visits ($75 for visit 1 [clinical interview, neuropsychiatric assessment, specimen collection] and $50 for visit 2 [specimen collection, neuroimaging evaluation]). Subjects who participate in the 6-month follow-up visits will receive an additional $125 ($75 for visit 3 [neuropsychiatric assessment, specimen collection] and $50 for visit 4 [specimen collection, neuroimaging evaluation]). In sum, subjects can earn $250 total if they complete all of the scheduled visits.

Enrollment procedures. Prior to enrollment, a phone screening will be conducted by study staff to ensure that Veterans are interested in participating and meet eligibility criteria. Following screening, interested Veterans will be scheduled for a first study visit to review consent materials either in person at the VAPORHCS, by telephone, or VA approved teleconferencing software (i.e., VA Video Connect [VVC] or non-public facing technologies [i.e., Cisco WebEx, Skype for Business, Zoom for Healthcare, Doxy.me]).

If the Veteran chooses to complete the first study visit by telephone or video, Veterans will be mailed informed consent materials (VA Informed Consent form, OHSU Informed Consent form, and VA HIPAA Authorization) and first visit materials (questionnaires). After the Veteran receives the study materials, a trained study team member will call the Veteran to go through the informed consent procedures and answer any questions. If the Veteran chooses to participate, he or she will sign the VA Informed Consent form, OHSU Informed Consent form, and VA HIPAA Authorization, and mail them back to Dr. Loftis using a pre-addressed and stamped envelope. Immediately after signing the consent forms, participants will have the option to complete portions of the neuropsychiatric study

visit measures, which includes the clinical interview, neuropsychiatric interview, neuropsychological assessment, and questionnaire battery via telephone or videoconference. Twelve of the fourteen neuropsychological assessments can be administered by phone or video. The remaining neuropsychological assessments will be administered at the time of the participant's next in-person visit. This research data will be collected from the participant prior to the study team receiving the signed informed consent forms and HIPAA authorization. Remaining research data and specimen collection will occur at the time of their next in-person research study visit once the signed informed consent forms and HIPAA authorization have been received.

Neuropsychiatric study visits. Clinical research staff will complete a standardized neuropsychiatric study visit protocol with eligible participants who provide informed consent. The protocol will be conducted twice for each participant (baseline and 12 weeks after DAA therapy completion).

The protocol includes the following sources of material: 1) Breathalyzer and/or saliva alcohol test (if subject presents at study visit in an apparent intoxicated state and/or study staff have any concerns about recent alcohol use) to provide estimates of blood alcohol levels and CIWA-Ar test results to provide estimates of alcohol withdrawal symptom severity, 2) Clinical interview to collect demographic data and medical [including liver health status (*e.g*., fibrosis) and virological information (*e.g*., HCV genotype)], medication (including prior exposure to IFN-based or other antiviral therapies), and substance use history, 3) SCID-5-RV to confirm past and present substance use disorders and psychiatric diagnoses, 4) medical record review to cross-validate the psychiatric, substance use, and medical history gained through clinical interview, 5) a neuropsychological battery to measure attention, memory, and executive function, 6) a questionnaire battery to measure neuropsychiatric symptoms (*e.g*., depression, anxiety, fatigue and pain), 7) blood, urine, and oral fluid collection for completion of medical laboratory tests [*i.e*., Blood is used for complete blood count (CBC), liver function panel, and HCV viral counts (obtained as part of standard care for HCV treatment). Oral fluid is used toward HIV antibody screening and alcohol testing. Urine is used for urine alcohol [*i.e*., ethyl glucuronide (EtG) levels] and drug analysis and pregnancy tests.], 8) blood sample collection for planned experiments and for contribution to the VA Liver Disease Repository (Director: Dr. Lissi Hansen), and 9) neuroimaging data [diffusion tensor imaging (DTI) and resting state and functional MRI scans are obtained at the Advanced Imaging Research Center (AIRC) at Oregon Health & Science University (OHSU) and the data are downloaded to a computer located at the VA Portland Health Care System (VAPORHCS)].

Veterans will have the option to complete portions of the neuropsychiatric study visit measures, including the clinical interview, neuropsychiatric interview, neuropsychological assessment, and questionnaire battery via telephone, videoconferencing, and/or mail. If a Veteran chooses to complete activities via telephone, video, and/or mail, a study team member will mail the Veteran the required visit materials (along with a pre-addressed and stamped envelope) and Dr. Loftis or another trained, IRB-approved study team member will conduct the activities via telephone or video.

If a Veteran chooses to complete the neuropsychological assessments via telephone or video, a study team member will administer the cognitive tests suitable for phone or video administration by reading the instructions and questions to the Veteran and recording their response. This will include twelve of the fourteen neuropsychological assessments. The remaining neuropsychological assessments will be administered at the time of the participant's next in-person visit.

Breathalyzer and saliva alcohol testing. A breathalyzer and/or saliva alcohol test is administered to participants that present at study visits in an apparent intoxicated state and/or study staff have any concerns about recent alcohol use in order to assure that participants are not impaired and to provide estimates of blood alcohol concentration (BAC) levels. The following test guidelines are used for our current and previous clinical studies in patients with AUDs. Test result = 0.00-0.05: Continue with

appointment; Test result > 0.05 but ≤ 0.08: We are not allowed to consent a person for participation in a research study if there is any indication that their thinking may be impaired. If reasonable, given time of day and BAC level, reschedule for later in the day. If a patient is allowed to return to retest later in the day, the original BAC should be recorded on the Screen Result form. Otherwise, reschedule for a future date; Test result > 0.08 (or patient is visibly impaired): visits are discontinued and follow-up procedures are conducted, including notifying the responsible clinician (Dr. Chang, Co-I). Participants that are over the legal limit will be rescheduled, and asked how they plan to get home. If taking public transportation, they will be allowed to leave unless clearly unable to take care of themselves based on clinical assessment of the responsible clinician. If driving in an automobile or motorcycle, or other personally controlled vehicle, they will be informed that they must remain at the hospital until they are no longer too inebriated to drive, will be retested using the breathalyzer or saliva alcohol test and recommended to stay in the clinic until the test is below the legal limit. If the participant threatens to leave and drive home while intoxicated, the VA Police will be called.

Alcohol withdrawal status. The Clinical Institute Withdrawal Assessment for Alcohol, revised version (CIWA-Ar) will be administered and scored at every visit. Using this scale mild alcohol withdrawal is defined with a score less than or equal to 15. For participants who score in the mild range, we will record the score and continue with the visit. For participants in the moderate (scores of 16 to 20) or severe (scores > 20) ranges, visits are discontinued and follow-up procedures are conducted, including notifying the responsible clinician (Dr. Chang, Co-I). Visits that are discontinued do to moderate or severe CIWA-Ar scores will be rescheduled.

Description of neuropsychiatric study visit measures. The neuropsychiatric study visit battery was designed to assess a full range of neuropsychiatric symptoms (*e.g*., AUD severity, cravings, depression, anxiety, fatigue, pain) and cognitive skills (*e.g*., attention, memory, executive function). The cognitive domains were selected for assessment because previous research by our group and others has demonstrated that HCV is associated with impairments in these domains (Huckans et al., 2009, Huckans et al., 2011). The present study follows up on these findings by evaluating whether individuals who receive DAA therapy for HCV evidence improved psychiatric and cognitive function, and the extent to which AUDs impact outcomes. Measures were selected because of their sound psychometric properties (*e.g*., reliability, validity, large normative sample that can be corrected for demographics), and because they are widely used by clinicians and have established clinical utility. *The neuropsychological measures that will be repeated have equivalent versions that will be alternated across study visits to minimize practice effects*. Measures will be administered according to published standard administration procedures.

Psychiatric and substance use disorder diagnoses

- SCID-5-RV. The SCID is a copyrighted and commercially available structured clinical interview. The SCID is administered via computer or in paper-pencil form and is also available in an abbreviated format [Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 2006)]. The interviewer asks participants about whether or not they have experienced specific psychiatric symptoms across their lifetime or recently in order to determine whether they have met DSM5 criteria for psychiatric and substance use disorders. Total administration time ranges from 15-60 minutes depending on the number of symptoms an individual endorses.

Neuropsychological battery

- Wide Range Achievement Test, Fifth Edition (WRAT5™) (Wilkinson and Robertson, 2017) This copyrighted and commercially available assessment to measure reading, spelling, and math skills is used to estimate baseline intellectual functioning. This measure will be administered at baseline only.

- Reynolds Intellectual Screening Test (RIST) (Reynolds and Kamphaus, 2003). This copyrighted and commercially available measure is used to estimate baseline intellectual functioning. This measure will be administered at baseline only.
- Neuropsychological Assessment Battery (NAB) (Stern and White, 2003). This copyrighted and commercially available battery of subtests assesses a variety of cognitive domains. We will administer the modules on attention, memory, and executive functioning at both study visits (*i.e.*, baseline and 6-month follow-up).
    - The Attention Module includes the following subtests:
        - Digit Span – measure of auditory attention capacity and working memory
        - Dots – measure of visual attention capacity and working memory
        - Numbers & Letters – measures of sustained visual attention, speeded information processing, and inhibition/switching
        - Driving Scenes – measure of visual attention/working memory
    - The Memory Module includes the following subtests:
        - List Learning – verbal learning and memory task
        - Shape Learning – visual learning and memory task
        - Story Learning – verbal learning and memory task, involving immediate and delayed recall of verbally presented stories
        - Daily Living Memory – verbal learning and memory task, involving recall of medication instructions, and a name, address, and phone number.
    - The Executive Functions Module consists of the following subtests:
        - Mazes – measure of problem-solving and planning
        - NAB Judgment - measure of everyday problem-solving/executive function
        - Categories – measure of mental flexibility and categorization
        - Word Generation – measure of initiation and mental flexibility.

Questionnaire battery. AUD Identification Test-C [AUDIT-C; 3-item screening tool to be administered at baseline only (Bush et al., 1998)]; Severity of Dependence Scale (SDS) (Gossop et al., 1997, Gossop et al., 1995); Alcohol Timeline Followback (TLFB) (to be administered in-person at pre- and post-DAA therapy study visits, as well as over the phone at weeks 12 and 18). In addition to in-person interviews, the TLFB has demonstrated reliability and validity over the phone (Sobell, 1992, Sobell et al., 1996); Clinical Institute Withdrawal Assessment for Alcohol scale, revised (CIWA-Ar) (Sullivan et al., 1989); Visual Analogue Scale (rates level of craving for alcohol over the past 24 hours on a scale of 0 to 100); Beck Depression Inventory, Second Edition (BDI) (Beck, 1996); Generalized Anxiety Disorder Inventory (GADI) (Argyropoulos et al., 2007) and PCL-C (Weathers et al., 1994); Fatigue Severity Scale (FSS) (Krupp et al., 1989); Brief Pain Inventory, Short Form (BPI) (Cleeland and Ryan, 1994), and Sickness Impact Profile (Bergner et al., 1981).

Specimen collection and laboratory measures. At study visits, oral fluid is collected and used toward HIV antibody screening by OraQuick (OraSure Technologies Inc.) or similar "in-home" oral swab test and alcohol testing by Alco-Screen (Chematics, Inc.) or similar alcohol saliva test. Urine is collected and used for urine alcohol [i.e., ethyl glucuronide (EtG) levels] and drug analysis and pregnancy tests. EtG assessments are done with EtG Alcohol Test Dip Cards (Rapid Exams Inc.). Drug analysis and pregnancy tests are conducted using 5 panel Drug Screen Cups (Rapid Exams Inc.) and human chorionic gonadotropin (hCG) urine dip card tests (Rapid Exams Inc.), respectively. Blood is collected by a trained phlebotomist or trained VA research personnel in four, 8 mL capacity Cell Preparation Tubes (CPTs, BD Biosciences) for planned experiments (≤ 32 mL of blood is collected at both the initial and 6-month study visits). If not already obtained per standard care for HCV, blood samples (≤ 23.5 mL) may also be collected to obtain HCV viral load, liver enzyme levels,

and complete blood counts by the VAPORHCS laboratory, per SOP. For participants without HCV and who do not require HCV viral load clinical laboratory test per standard of care, absence of HCV will be confirmed using Oraquick HCV rapid antibody test (OraSure Technologies Inc.). Following the neuropsychiatric study visit, research staff immediately transports the human blood samples to Dr. Loftis' laboratory for processing and storage. Dr. Loftis' research staff centrifuges the blood samples per SOPs so that components [*e.g.*, plasma, peripheral blood mononuclear cells (PBMCs)] can be collected, frozen, and cryopreserved.

Immune factor detection. Plasma samples and cell culture supernatants will be analyzed for the expression of specific immune activation markers using multiplex immunoassays and Luminex technology and PCR [*e.g.*, (Wilhelm et al., 2013, Wilhelm et al., 2012)]. The immune activation profile [*i.e.*, CRP, interferon-gamma (IFN-γ)-inducible protein-10 (CXCL10), IL-1β, IL-6, IL-8, IL-10, IL-18, MCP-1, MMP-3, S100B, TIMP-1, TNF-α] was selected based on published (Huckans et al., 2014, Loftis et al., 2008, Mascia et al., 2017) and preliminary findings (**Figs. 3 and 4**) regarding the role of these cytokines and chemokines in infection control and neuropsychiatric impairments.

Immune cell phenotype and function. PBMCs collected before and after DAA therapy will be used to evaluate phenotypic and functional changes in memory T cells (central, effector, and transitional) and macrophages that regulate viral suppression and persistence. Cell phenotype will be determined using flow cytometry, as previously described [*e.g.*, (Loftis et al., 2016, Wilhelm et al., 2013)]. Briefly, single-cell suspensions of human PBMCs will be stained to identify and analyze viable cell phenotypes, including subsets of CD4+ and CD8+ T cells (*e.g.*, effector and regulatory cell subpopulations), programmed cell death 1+ (PD-1+) cells, and activated macrophages ($CD45^{hi}CD11b+$ cells). Immune cell function will be assessed by measuring cell proliferation and inflammatory factors secreted from cells stimulated *in vitro*, as described in methods reviewed by the VA Subcommittee on Research Safety (SRS). Given that chronic viral infections and AUD are associated with phenotypic and functional impairments in CD4 and CD8 T cell subsets and the PD-1 inhibitory receptor [measure of an "exhausted" phenotype; *e.g.*, (Utzschneider et al., 2016)], we will focus on these immune cells and signaling pathways. Our analyses will additionally be guided by a recent cross-sectional study of patients with AUD which found significant alterations in CD8+ and CD4+ T subpopulations in patients with AUD, as compared to healthy controls (Zuluaga et al., 2016).

Neuroimaging.* Following the neuropsychiatric study visit, all eligible participants are scheduled for a separate neuroimaging study visit. Neuroimaging may be omitted for medical reasons or if determined by study team to not be in the subject's best interest. Subjects, well characterized with respect to their substance use, will then be evaluated with fMRI tasks (BART and MID), rsMRI, high resolution anatomical MRI, standard DWI and high angular resolution diffusion imaging (HARDI). Breathalyer tests, alcohol withdrawal symptom scales, urine alcohol and drug screens, and pregnancy tests will be administered at the neuropsychiatric study visits and the imaging evaluations. The neuroimaging protocol will take approximately one hour and will include the Balloon Analogue Risk Task (BART) and Monetary Incentive Delay Task (MID).

Balloon Analogue Risk Task (BART): A version of the BART (Lejuez et al., 2002), adapted for event-related fMRI, will be used. Balloons are either red or blue (active trials) or white (control trials). When presented with an active balloon, participants will select between pumping the balloon for a potential increase in earnings ($0.25/pump) or cashing out to retain earnings accumulated during that trial. Continued pumping will ultimately result in a balloon explosion and the forfeiture of unrealized earnings accumulated during the trial. Prior to scanning, participants will be informed that red and blue balloons are associated with monetary reward but that they will not receive the monetary reward at the end of the trial. Participants will not be informed that the number of pumps to produce an explosion is pre-determined for each balloon from a uniform probability distribution (maximum 8 or 12

pumps for red and blue balloons, respectively). Participants will be informed that the white balloons do not explode and are not associated with potential reward, and they will be instructed to pump the white balloon until the end of the trial (maximum 12 pumps according to a uniform probability distribution). The task will be administered in two 10-min runs; typically 50-60 active trials and 10-14 control trials. Participants will not receive the earnings at the end of the scanning session. The variables to assess adaptive decision-making and risk-taking behavior include: the total and average number of pumps across all trials and in trials that end in a choice to cash-out ("adjusted pumps"), as balloon explosions may restrict the range of risk-taking behavior; the total and average number of cash-out trials, total earnings, and the average reaction time (RT) for all pumps, cash-outs, and of the first and last pump from each trial.

Monetary Incentive Delay Task (MID): The MID is a validated task to examine incentive-reward reactivity of the ventral striatum. The task requires little cognitive demand and participants simply respond to a brief presentation of a visual target to win or lose money. Participants will not receive the winnings from this task. The MID task session consists of 180 6-second trials. During each trial, participants are shown a cue (cue phase, 1 second), indicating that they have the potential to win or lose money ($0.20, $1 or $5) or that no money is at stake for that trial (Control). A fixation cross is present during a variable interval (anticipatory delay phase, 2000–2500 ms), and when a target appears, participants press a button (target phase, 160–260 ms). For WIN trials, participants will be told that if they successfully press the button while the target is onscreen (a "hit") they will win the amount of money indicated by the cue. For LOSE trials, participants will be told that no money will be won or lost for hits, but misses will lead to a loss of the amount indicated by the cue for that trial. By contrasting brain activations in the anticipatory delay phase in WIN trials vs. Control trials it is possible to examine the extent to which the anticipation of potential reward modulates ventral striatal activity during this preparatory period. A Feedback phase (1650 ms) follows the target's disappearance. Feedback will display amount won or lost for each trial and total cumulative winnings.

**Methodological details for imaging acquisition, processing, and analysis were provided by the Hoffman lab and are summarized on pages 10-11.*

MRI Imaging Acquisition: We will use the Human Connectome Project (HCP) Lifespan Protocol using a 3T Siemens Magnetom Prisma scanner and a 32-channel phased array head coil. **fMRI (T_2*-weighted):** Three (2 PDD and 1 DSNBACK) T_2*- weighted echo-planar imaging (EPI) functional runs will be acquired (60 slices, 2 mm thick, TR/TE/α=2000 ms/38 ms/55°, matrix=128 × 128, FOV=220 mm^2, PAT mode/Accel factor = GRAPPA/2, 310 volumes per run) with an in-plane pixel size of 2.0 mm^2 while subjects perform the PDD or the DSNBACK. Each run will be 10.3 minutes long. **rsfMRI**: Identical to fMRI settings with 200 volumes. **High-resolution anatomical (T_1-weighted [T_{1w}]):** One magnetically prepared rapid acquisition gradient echo (MPRAGE) (208 0.8 mm isovoxels, TR/TE/TI/α = 2400 ms/2.24 ms/1060 ms/8°, FOV=256x240 mm, 2x Parallel Imaging, duration 6.38 min), will be acquired for co-registration with functional images and statistical overlay. **High-resolution anatomical (T_2-weighted [T_{2w}]):** 208 slices, 0.8 mm isovoxels, TR/TE: 3200 ms/564 ms; variable flip angle; FOV 256x263 mm, 2x Parallel imaging; Futsyion 5.57 min. **HARDI:** Two HARDI scans will be acquired (66 axial slices, 2.2 mm thick, TR/TE/α = 4300 ms/96 ms/90°, FOV= 25.6 cm^2, PAT mode /Accel factor = GRAPPA/2) consisting of six non-diffusion weighted (B_0) images followed by 32 non-collinear directions at B_0 = 1000 s mm^{-2} and at B_0 = 2000 s mm^{-2}. Each HARDI scan lasts 4.73 min.

fMRI Standard Preprocessing: Task-related and resting-state fMRI data will be preprocessed identically. We include only scans with minimal movement (< 3 mm) and free of artifacts (Murphy et al., 2013). Functional images will be realigned for motion corrections, unwarped then co-registered to structural images for each subject and then transformed to Talairach coordinates and then resampled into atlas space with 3 mm isovoxels. Motion correction is applied with the standard 6 parameters and then frame-by frame spatial deviations are assessed with the temporal derivative of the time course.

The anatomical volume is then segmented into gray matter, white matter, and cerebrospinal fluid. Next, we apply a temporal bandpass filter (0.009 Hz < ν < 0.08Hz), spatial smoothing (6 mm full width at half maximum (FWHM) Gaussian kernel), regression with the 6 rigid body motion parameters, regression of the whole brain signal and regression of the signal for ventricles and white matter. Opposite phase-encode polarity spin echo images will be used to estimate the B_0 distortion field, which will be applied to each gradient echo frame after accounting for estimated head motion. Gradient nonlinearity distortions will be corrected using scanner-specific field maps. Both corrections are vital for proper registration to structural images. Images will be preprocessed using AFNI_16.1.26. To enable cortical surface-based analyses, fMRI time courses will be sampled to the cortical surface mesh, using the extent of overlap with the cortical ribbon as a weighting factor to account for partial voluming. Voxels with high variability relative to their local neighborhood will be excluded. The functional EPI images will be despiked (3dDespike), slice time corrected (3dTshift), motion corrected (3dvolreg), aligned to warped MPRAGE (align_epi_anat.py) and corrected for oblique slice acquisition. A 4 mm FWHM three-dimensional Gaussian blur will be applied to the functional images (3dmerge) and each voxel time series will be scaled to have a mean of 100 (3dTstat, 3dcalc).

Anatomical (T_{1w} and T_{2w}). MPRAGE and T_{2w} structural images will be corrected for gradient nonlinearity distortions using scanner specific, nonlinear transformations, provided by MRI scanner manufacturers, and verified using phantom scans. Spatial distortion in the readout direction due to B_0 variation will be corrected based on the distortion maps generated from our fast field mapping. T_{2w} images will be registered to T_{1w} images using mutual information after coarse pre-alignment via within-modality registration to atlas brains. Intensity non-uniformity correction will be performed by dividing image intensities by a smooth bias field estimated from the geometric mean of the T_{1w} and T_{2w} images. Anatomical scans will be corrected for oblique acquisition, skull stripped (3dSkullStrip), and warped into MNI-152 space with @auto_tlrc.

Diffusion-Weighted Imaging: DTI preprocessing will be performed using the DTI preprocess script (Takuya Hayashi, Center for Life Science Technologies, RIKEN, Kobe, JP) for FSL [(Smith et al., 2004) FMRIB, Oxford]. Scans will be corrected for eddy current distortion and motion. B0 inhomogeneity distortion will be corrected using a B0 field map (magnitude and phase) in FSL's fugue. DTI estimates (FA, $\lambda_{1\text{-}3}$, $V_{1\text{-}3}$, MD) will be calculated using DTIFIT. Voxelwise statistical analysis will be performed with TBSS (Smith et al., 2006) and nonlinear registration to the FMRIB58_FA common space will be conducted. Each subject's aligned FA data will be projected onto a mean skeleton. Voxelwise cross-subject statistics will be performed with FSL's *Randomise* with threshold-free cluster enhancement (TFCE).

Voxel-Based Morphometry: Structural images will be analyzed with FSL-VBM (Douaud et al., 2007) an optimized VBM protocol (Good et al., 2001) carried out with FSL (Smith et al., 2004). Images will be brain-extracted and tissue segmented, registered to the MNI-152 standard space using non-linear registration (Andersson et al., 2007), and flipped along the x-axis to create a left-right symmetric, study-specific gray matter template and modulated with a voxel-wise division by the Jacobian determinant of the non-linear transformation matrix to correct for local expansion or contraction. The modulated gray matter images will be smoothed with an isotropic Gaussian kernel with a sigma of 3 mm (FWHM = 7 mm). Group contrasts will be tested with FSL's *Randomise*, a permutation-based non-parametric procedure.

Statistical analyses and power. Statistical analyses for the project will be performed by the research team and the Biostatistics and Design Program at OHSU. All data sent to OHSU for statistical analysis will be coded.

Neuropsychiatric and laboratory measures. We plan to calculate the change in the response over six months between post- and pre-DAA therapy for each subject and use general linear models (GLMs) to assess whether average change differs among the four groups—either due to AUD, HCV, or the

potential interaction of these factors—after possibly controlling for other subject-level covariates that remain constant (or nearly so) over the six months (*e.g.*, age, sex, HCV genotype). Variables that could meaningfully vary over time (*e.g.*, TFLB score, CIWA-Ar score) will be summarized in terms of a difference and then included as a covariate. Number of DAA therapy provider contacts will be included as an extra covariate that counts total contacts. All models will use "robust" (sandwich estimators) of variance to guard against potential misspecification of the underlying distribution of change scores or due to non-constant variance among the groups (Long and Ervin, 2000). The AUD × HCV interaction will be assessed first in all models fit, and will be removed from the model only if evidence suggests ($p > 0.10$) these two factors don't modify one another; otherwise, subsequent comparisons of each separate effect will be made within each level of the other (*e.g.*, effect of HCV separately characterized for those with and then without AUD).

A supervised and directed approach will be taken in the exploratory analysis of inflammatory biomarkers. Principal component analysis (PCA) will be used to reduce the collection of ~12 correlated immune factors to a smaller number of uncorrelated linear combinations (the principal components) of biomarkers [*e.g.*, (Bowman et al., 2012)]. Each component of this reduced set of uncorrelated patterns of inflammatory expression will be subjected to the same general linear models described earlier to determine whether AUD, HCV or AUD x HCV are associated with it, even after adjustment for other covariates. If the overall test of association in the linear model shows significant ($p < 0.10$, for initial screen) variation among the groups, then separate models will be conducted on those biomarkers that most heavily contribute/dominate the principal component being screened. In this way, we may identify combinations of markers that are sensitive to AUD and HCV treatment outcomes, particularly CNS function.

Neuroimaging measures. We will use the methods of the Fair Laboratory (Miranda-Dominguez et al., 2014) to analyze the rsMRI scans. We will use an ROI-to-ROI approach to calculate connectivity in the pre-processed data, based on the a priori regions previously associated with alcohol effects. Bivariate correlations between the average (BOLD) signal from these regions provide connectivity estimates. Several sources of spurious variance along with their temporal derivatives are then removed from the data through linear regression (*e.g.*, signal from ventricular regions and signal from the white matter). The magnitude of connectivity changes between groups will be compared utilizing two-tailed t-tests. The raw p-values will be corrected for multiple comparisons and thresholded at a false discovery rate (FDR) correction of $PFDR < 0.05$. Data from discounting tasks will be fit to equations using a softmax procedure (Miedl et al., 2012). We calculate the fractional areas under the discounting curves as impulsivity measures.

Power. We estimated group size using fmripower (Mumford and Nichols, 2008). The MATLAB-based program calculates a power estimate given the between-group and within-subject error terms associated with a two tailed t-test of regression coefficients. The results suggested that 30 subjects (male and female) per group would yield a power of 0.8 for the test comparison. This sample size yields an 80% chance for us to identify AUD × HCV interaction effects of 0.91 standard deviation (SD) or larger at the 0.10 level of significance. Use of the higher alpha-level is done to lessen the chance of making a type II (overlook) error and mistakenly omitting this term from the model. We also have 80% power for a 0.05-level test to detect any main effect, for either AUD or for HCV, of at least 0.51 SD in size. **Table 2** shows the smallest average change (between post- and pre-DAA therapy) among groups that would be of practical interest and the subsequent main effects (AUD+ vs AUD-; HCV+ vs HCV-) and interactions that derive from these minimally relevant average changes. The anticipated SD for the change scores was derived from reported SDs of each outcome supported by literature, and then converted to a SD for the difference assuming a modest correlation of 0.60 between pre- and post-DAA responses within the same subject. The principal comparisons proposed in this work parallel those in our preliminary work, and we were able to readily demonstrate differences in planned comparisons between the groups. Therefore, the proposed 30 subjects per group should provide us with sufficient power to test the predictions, anticipating that a small number of participants with HCV may not achieve SVR (n = ~3-6).

Table 2. Statistical power to evaluate antiviral therapy outcomes of significant clinical impact

		Treatment group[a]				Effects and Power[b]		
Response/outcome	SD(diff)	AUD-/HCV-	AUD-/HCV+	AUD+/HCV-	AUD+/HCV+	AUD main	HCV main	Interact.
Cognitive performance[c]								
Attention	2.25	3.5	3.7	1.2	3.5	-1.25 (0.85)	1.25 (0.85)	2.1 (0.81)
Memory	2.50	3.0	3.4	0.4	3.0	-1.50 (0.91)	1.50 (0.91)	2.2 (0.78)
Executive function	1.80	2.0	2.1	0.3	2.0	-0.90 (0.78)	0.90 (0.78)	1.6 (0.78)
Behavioral response								
Impulsivity (BART/MID)[d]	1.35	0	-0.2	-0.1	-1.5	-0.70 (0.80)	-0.80 (0.90)	-1.20 (0.78)
Alcohol use (TLFB)	18.9	0	-1.0	-18	-36	-26.5 (0.99)	-9.50 (0.78)	-17.0 (0.79)
Mental health								
Depression (BDI)	4.0	0	-3.5	-2.7	-11.0	-5.1 (0.99)	-5.9 (0.99)	-4.8 (0.94)
Fatigue (FSS)	0.63	0	-1.1	-0.8	-1.3	-0.5 (0.99)	-0.8 (0.99)	0.6 (0.83)
CNS microstructure[e]								
FA	0.02	0	-0.001	-0.003	-0.02	-0.01 (0.91)	-0.01 (0.78)	-0.016 (0.78)
MD (x10^5)	8.9	0	-6.0	7.5	15.0	14.5 (0.99)	0.75 (0.07)	13.5 (0.99)
Inflammatory profile								
Immune factor concentration[f]	4.05	0	-10	2.5	-3	4.75 (0.99)	-7.75 (0.99)	4.5 (0.92)
T cell subpopulations[g]	22.5 DP 27.0 DN	0 0	-18 DP 38 DN	0 0	-9 DP 19 DN	4.5 (0.19) -9.5 (0.48)	-13.5 (0.90) 28.5 (0.99)	9 (0.29) -19 (0.61)

[a]Numbers shown are minimally meaningful mean change scores, post- minus pre-DAA therapy, for the primary outcome variables. Conjectured standard deviation for a given outcome was based on published studies conducted by the PI and others, as referenced and converted to a standard deviation (SD) for the difference by conservatively assuming a correlation of 0.60 between post- and pre-DAA therapy values. [b]Power for main effects use 0.05 as level of significance while interaction uses 0.10. Based on n = 30 per group (N = 120 total). [c]NAB module scores are reported as demographically corrected standard index change scores. [d]Higher (*i.e.*, less negative) values represent a stronger tendency to discount larger delayed rewards in favor of smaller immediate rewards. [e]Neuroimaging outcome projections are for brain regions and tracks most significantly affected by a history of HCV infection and alcohol abuse (*e.g.*, insula, superior fronto-occipital fasciculus). [f]Immune factors included in the inflammatory profile: CRP, CXCL10, IL-1β, IL-6, IL-8, IL-10, IL-18, MCP-1, MMP-3, S100B, TIMP-1, TNF-α. [g]T-cells will initially be evaluated for changes in the frequencies of double negative (DN; CD4-CD8-) and double positive (DP; CD4+CD8+) CD3+ cells; further investigation of subpopulations (*e.g.*, effector, regulatory) will be conducted as described under Laboratory measures and in the Specific methods section. Abbreviations: BDI = Beck Depression Inventory, 2nd Edition; CNS = central nervous system; DAA = direct acting antiviral therapy; BART/MID = Balloon analogue risk task/Monetary incentive delay task; FSS = Fatigue Severity Scale; FA = fractional anisotropy; MD = mean diffusivity; NAB = Neuropsychological Assessment Battery

Expected outcomes and alterative considerations. Results are expected to determine the extent of improvement in CNS function (including neuropsychiatric outcomes) that is gained by successful completion of DAA therapy and whether or not co-morbid AUDs affect HCV treatment outcomes. Our hypotheses would be supported by finding improved CNS function following successful completion of DAA therapy (*i.e*., obtaining an SVR), including: i) increased FA in the tracts that previously connected regions between which there was decreased functional connectivity, ii) improved functional connectivity, iii) reduced impulsivity, and iv) improved cognitive abilities. Following DAA therapy, these changes in CNS function will be associated with reduced peripheral immune activation. Individuals with active AUDs and HCV may show attenuated benefits in CNS function and immune response, post-DAA therapy. In participants with HCV and co-morbid AUDs, cumulative disruptions in cerebral connectivity that result from anatomical disruptions may contribute to persistent cognitive deficits and elevated inflammatory profiles. Alternatively, it is possible that floor effects for some of the variables (*e.g*., BDI-II scores) may limit our ability to detect significant improvements. However, cognitive performance benefits have been reported following IFN-based therapy for HCV (Kraus et al., 2013, Kuhn et al., 2017), and we have observed improvements in depression and fatigue in patients who obtained SVR (Huckans et al., 2015). It is also possible that differences in the number of DAA therapy provider visits between participants in the HCV+ and HCV- groups may affect neuropsychiatric outcome variables (*e.g*., alcohol use behavior). To assess the impact of this potential confound, we plan to include that characteristic as an extra covariate. Because CNS recovery may continue beyond the SVR assessment timepoint, patients who agree to participate in the study will be asked to allow our research team permission (via the informed consent process) to contact them for future follow-up investigation, which is beyond the scope of this current protocol. We also considered the potential concern that there may be a lack of research participants with HCV (given the pace at which the VA is treating HCV); however, estimates indicate that by 2020, there will still be ~560,000 adults with HCV in the US (Chhatwal et al., 2016).

Table 3. Project timeline

Year 1	Year 2	Year 3	Year 4
To test hypotheses for aims 1 and 2, 120 Veterans will be recruited to participate in a prospective study that will evaluate patients with HCV before and after DAA therapy. Participants will be enrolled in the study for a total of six months			
Months 1-3: Obtain IRB and other regulatory approvals; Apply for Certificate of Confidentiality; Train staff in protocol and data analysis.	Months 13-15: Enroll and conduct baseline study visits; Complete 6-month follow-up visits for ~10 participants	Months 25-28: Complete 6-month follow-up visits for ~10 participants.	Months 37–40: Complete 6-month follow-up visits for remaining participants (*goal: n = 120 completed by month 40*); Conduct flow cytometry analysis of cryopreserved PBMCs.
Month 4: Begin recruitment of subjects using existing relationships with clinics at the VAPORHCS; Enroll and conduct baseline study visits for 4 participants. Months 5-9: Enroll and conduct baseline study visits for 30 participants at a rate of ~1-2 participants per week.	Months 16-19; Enroll and conduct baseline study visits; Begin analyzing behavioral data and continue to conduct analysis of individual neuroimaging data; Complete 6-month follow-up visits for ~14 participants.	Months 29-35: Complete 6-month follow-up visits for ~30 participants; Assess drop-out rates and intensify follow-up efforts, if needed.	Month 41-42: Perform multiplex plasma sample testing for immune activation biomarkers; Submit abstract for conference presentation.
Month 10: First 4 participants complete study procedures; Begin preliminary analysis of individual neuroimaging data; Enroll and conduct baseline study visits. Months 11-12: Enroll and conduct baseline study visits for 10 participants; Complete 6-month follow-up visits for ~10 participants; Assess recruitment and drop-out rates and intensify follow-up efforts, if	Months 20-24: Enroll and conduct baseline study visits; Complete 6-month follow-up visits for ~16 participants; Assess recruitment and drop-out rates and intensify follow-up efforts, if needed (*goal: n = 60 completed by end of year 2*); Submit abstract for conference presentation.	Month 36: Complete 6-month follow-up visits (*goal n = 100 completed by end of year 3/beginning of year 4*); Continue to analyze behavioral and neuroimaging data; Submit abstract for conference presentation; Compile results for future publications (*see months 43-48*), and if sample sizes permit, publish behavioral findings (outcomes critical for patients with HCV seeking DAA therapy).	Months 43-48: Complete human subjects data analysis; Prepare and submit final manuscripts. Disseminate evidence-based information regarding the effects of AUD on DAA therapy outcomes, as appropriate

needed (*goal: n = 20 completed by end of year 1*).			

Study Population

Up to 150 participants will be consented for this study (including screen failures), and 120 participants will be enrolled (n = 30 per group). **Table 3** summarizes a time table for the overall study.

Standardized inclusion and exclusion criteria. Adult Veterans (≥ 21 years old) who are about to initiate DAA therapy for HCV are recruited into two demographically matched (*i.e*., similar in terms of age, gender, race, and years of education) groups (n = 30/group): 1) adults with current alcohol use disorder (AUD+/HCV+), confirmed with the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), Research Version (SCID-5-RV), and 2) adults without current AUDs (AUD-/HCV+), confirmed with the SCID-5-RV. Two additional groups of demographically-matched adults: 1) without AUD or HCV (AUD-/HCV-) (n = 30), and 2) with AUD and no HCV (AUD+/HCV-) will be recruited as a comparison groups to determine the relative contribution of a history of HCV infection to alcohol-induced brain pathology.

Exclusion criteria for the AUD+/HCV+ and AUD+/HCV- groups include: 1) medical conditions (other than HCV for the AUD+/HCV+ group) likely to impact immunological function or central nervous system function (such as HIV, cancer, lupus, stroke, neurodegenerative disease, hepatic encephalopathy, multiple sclerosis, or a traumatic brain injury), 2) visible intoxication or impaired capacity to understand study risks and benefits or otherwise provide informed consent (*note: subjects will be administered alcohol withdrawal tests and, if deemed necessary due to possible recent alcohol use, breathalyzer and/or saliva alcohol tests prior to each study visit*), 3) schizophrenia, schizoaffective disorder, or current psychosis or mania, 4) visual or auditory impairments that would prevent valid neuropsychiatric testing, and 5) contraindications to magnetic resonance imaging (MRI) (*e.g*., surgical aneurysm clips, pacemaker, prosthetic heart valve, neuro-stimulator, implanted pumps, cochlear implants, metal rods, plates or screws, previous surgery, hearing aids, history of welding, metal shrapnel, or potentially pregnant).

Exclusion criteria for the AUD-/HCV- and AUD-/HCV+ groups include: 1) medical conditions (other than HCV for the AUD-/HCV+ group only) likely to impact immunological function or central nervous system function (such as HIV, cancer, lupus, stroke, neurodegenerative disease, hepatic encephalopathy, multiple sclerosis, or a traumatic brain injury), 2) visible intoxication or impaired capacity to understand study risks and benefits or otherwise provide informed consent (*note: subjects will be administered alcohol withdrawal tests and, if deemed necessary due to possible recent alcohol use, breathalyzer and/or saliva alcohol tests prior to each study visit*), 3) schizophrenia, schizoaffective disorder, or current psychosis or mania, 4) heavy alcohol use (Women: average alcohol use > 7 drinks per week for ≥ one year. Men: average alcohol use > 14 drinks per week for ≥ one year.), 5) visual or auditory impairments that would prevent valid neuropsychiatric testing, and 6) contraindications to MRI (*e.g*., surgical aneurysm clips, pacemaker, prosthetic heart valve, neuro-stimulator, implanted pumps, cochlear implants, metal rods, plates or screws, previous surgery, hearing aids, history of welding, metal shrapnel, or potentially pregnant).

Subject Identification/Recruitment

Recruitment, eligibility screening, consenting, and neuropsychiatric study visits will be conducted by approved clinical research staff at the VA Portland Health Care System. A Waiver of Authorization and Informed Consent Process for Screening/Recruitment Purposes will be obtained to facilitate subject identification and recruitment.

1. Potential participants who meet the study criteria will be recruited into this study through word of mouth referrals from clinicians at VAPORHCS. Research staff may contact patients who

indicate to their clinicians that they are interested in participating. Potential participants may also contact the study personnel after learning of the study (e.g., from advertisements). Initial phone screening will be done for each subject when they contact the lab and are interested in participating. The potential participant will be screened by study personnel using a screening form. Potential participants will be recruited from the Substance Abuse Treatment Program (SATP) and Liver Clinic. A study member will give regular presentations of the study at SATP access meetings. Attendance at these recruitment meetings by potential subjects is entirely voluntary. Potential subjects will be given the IRB-approved advertisement with contact information and asked to call the contact number if they are interested in participation.

2. IRB-approved flyers will be posted in designated research advertisement space at VAPORHCS, which includes elevators and lobby spaces, as well as common spaces at OHSU and in the community.
3. Other recruitment strategies include the use of social media. Study advertisements will be posted on VAPORHCS sponsored social media accounts including internal publication/promotion (Weekly E-news employee newsletter and Employee SharePoint E-Post) and external publication/promotion (VA Social Media Sites including VAPORHCS Facebook and Twitter pages, Public VAPORHCS Web Page, Quarterly Veteran Connection Newsletter, and Email Updates through GovDelivery). All recruitment advertisements will ask prospective participants to telephone the study coordinator if they are interested in participating. Email or social media messaging will not be utilized. Craigslist will also be utilized for recruitment. The study coordinator will create Craigslist postings that include a text version of the IRB approved study advertisement. Email responses to the Craigslist posting will be disabled so subjects will only be able to respond via telephone. All potential participants will be screened using the screening form when they contact research staff.
4. We will conduct a CPRS search of patients who have been previously seen in the Gastroenterology and Hepatology and/or Mental Health & Clinical Neurosciences Division at the VA Portland Health Care System to identify patients >21 years old who have been diagnosed with HCV and/or an alcohol-use disorder. We will then conduct a Pre-Screening (i.e., via a medical record review) of individuals who meet the diagnostic criteria to identify individuals who meet study criteria. We will then send individuals who meet the study criteria an individualized letter (i.e., addressed to the patient) from the Clinical Director of the Mental Health & Clinical Neurosciences Division and/or the Section Chief of Gastroenterology and Hepatology about the study; the Director's letter will be accompanied by our study flyer and a form letter (not individualized) from the study PI. The letter will provide the Research Assurance Officer's telephone number so the potential participant can verify that the study constitutes VA research. The letter will state that the individual may opt out by calling the research team or returning an enclosed document indicating that they do not wish to be contacted (using a pre-stamped/addressed return envelope that we will provide), or they may indicate they are interested in learning more about the study by calling the study team or returning the enclosed document saying they want to be contacted. The letter will state that if the individual has not returned a response to the letter within two weeks, research personnel will contact them by phone. Study Personnel will call all those who are interested in the study to answer any questions they might have about the study and to schedule an Initial Study Visit if they remain interested. If no response to the letter is received within two weeks, the research team will contact the individual by phone but with limited interaction and using a phone script. The researcher will identify themselves, explain why they are calling, and ask if the individual received the letter. If they did, the researcher will ask if they have reviewed the letter and whether or not they are interested in hearing more about the study. If the individual has

received but not reviewed the letter, the researcher may ask if they are interested in hearing more about the study or receiving more information. After answering their questions, the researcher will schedule an Initial Study Visit for those who state they are interested in participating. If the Veteran has not received the letter, the researcher will only ask permission to send a second letter and confirm the individual's address; no other information will be requested at that time.

An appointment confirmation letter will be mailed to a participant after they have scheduled their initial visit. An appointment confirmation letter will also be sent prior to the week 24 visit. If a participant is enrolled in the study and not returning phone calls to schedule additional research visits, a no-show letter will be sent in the mail asking the participant to contact the research coordinator to discuss their interest in their continued involvement in the research study. If the research coordinator does not hear back from the participant within two weeks of sending the letter, the participant will be disenrolled from the research study.

Reminder phone calls will be made the day prior to scheduled on-site appointments. During reminder calls, study team will ask participants if they have recently had any recent exposure and/or symptoms of COVID-19. If a participant suggests that they might be experiencing symptoms or have had a known exposure to COVID-19, we will offer information on COVID-19 testing and self-isolation and postpone the study visit for at least two weeks following exposure or until symptoms are completely resolved. When the participant arrives for their appointment, their temperature will be taken using a digital thermometer and the same screening questions will be asked again before beginning the appointment. The participant will be sent home with the same information on testing and self-isolation upon indication or disclosure of any symptoms or exposure to someone with confirmed or suspected COVID-19. Also during confirmation calls, participants will be informed that masks are required to be worn during their entire visit. If a subject does not have access to a mask, the study team will provide one. Subjects will be instructed on how to properly care for their masks if they need to remove them during the study visit.

Inclusion of women and minorities. Inclusion of women and minorities in this prospective cohort study is appropriate and is encouraged. Subject selection is based only on the criteria described in the subject eligibility section. Women and minorities are not excluded from the study solely based on either of those demographic characteristics. Population characteristics, racial characteristics, and prior experience with recruitment at VA health care systems reveal that it is a good place to conduct such a study since the prevalence of HCV infection and co-morbid AUDs are disproportionately high among the Veteran population. Because sex effects may be observed, we will make every effort to enroll equal numbers of men and women—a charge that is less challenging than it previously was, as the number of female service members has been rapidly increasing over the last few years. It was recently reported that; "In 2013, female service members accounted for 14.9% of the Department of Defense active duty force and 18.5% of the selected reserve forces" (Department of Defense. 2013 Demographics: Profile of the military community; summarized in (Brickell et al., 2017)). Given that all research participants will be Veterans treated within the VAPORHCS, children will not be included in this study.

Informed Consent & HIPAA Authorization

Interested Veterans will be given the opportunity to meet in person at VAPORHCS or by telephone or video to complete informed consent procedures. If the Veteran chooses to complete the informed consent process by phone or video, study staff will mail materials to the Veteran along with a pre-addressed and stamped envelope. Study materials include: the VA Informed Consent form, OHSU Informed Consent form, and VA HIPAA Authorization. At the initial meeting, approved study

personnel will describe the study, apprise the participant of the risks, determine if the participant meets inclusion criteria and, if the participant wishes to participate, obtain written informed consent and HIPAA authorization. The participant will be given the opportunity to ask questions and discuss the protocol with their family. If the Veteran chooses to participate, he or she will sign the VA Informed Consent form, OHSU Informed Consent form, and VA HIPAA Authorization. If completed by phone or video, the participant will mail the documents back to Dr. Loftis using the pre-addressed and stamped envelope.

If consenting is completed remotely, study staff will offer the participant the opportunity to complete portions of the neuropsychiatric study visit measures, including the clinical interview, neuropsychiatric interview, 12 out of 14 neuropsychological assessment measures, and questionnaire battery via telephone or video. Thus, research data be collected prior to the signed informed consent form and authorization having been received by the study team. Remaining research data and specimen collection will occur at the time of the participant's next in-person research study visit once the signed informed consent forms and HIPAA authorization have been received.

Risks, Side Effects, and Protections

This human subjects prospective cohort study does not involve treatment or invasive procedures. The DAA therapy that patients will be receiving is part of standard care for HCV (and not administered for research purposes).

- Patients may become upset while answering personal questions during the clinical interview. There are also risks to confidentiality, particularly of sensitive information about drug use and abuse and HIV/HCV infection status.
- Subjects who experience claustrophobia may become anxious in the MRI instrument. Subjects with metal fragments, wire sutures, staples, implanted pacemakers or defibrillators or metal fragments from welding are at risk from damage due to movement of metal fragments in the 3T magnetic field. Participants will be systematically assessed for metallic foreign bodies and excluded if there are any uncertainties. The research team will obtain orbital x-rays if subjects have any history of welding, grinding or other exposure to metallic particles that could lodge in the orbit. Pregnant women (negative urine pregnancy test required before the scan), because of the unknown risk to the fetus, will also be excluded. Rarely, subjects experience vertigo, a metallic taste, or muscle stimulation. If a subject becomes anxious during the procedure, the procedure can be terminated immediately.
- Blood drawing may cause pain or bruising at the site and carries a small risk of infection.

Additional protections from risk. In order to provide additional protections against risk, during the consent process, subjects will be asked to sign an optional release of information to have laboratory test results released to their primary care provider, hepatologist, or other appropriate health care provider. In the event of abnormal results, clinical research staff will report the results to the participant and instruct the participant to consult with their health care provider to determine whether follow-up is required. Clinical research staff will also release results to the healthcare provider if the subject opted to sign a release during the study visit. Clinical research staff will have clinical experience and training (*e.g.*, clinical psychology, clinical research) and will be trained to provide pre- and post-test counseling specific to HIV testing and HCV testing. Clinical research staff will be available to subjects for a post-test counseling visit in the event of a positive screen.

A mental health provider (*e.g.*, Drs. Hoffman) will be available to consult with any participants who experience discomfort for any reason during the study visit. Subjects will be asked to remain at the study visit either until the problem has been resolved or referral to appropriate treatment is accomplished. Subjects not in need of emergent treatment will be offered referral to counseling or

other support resources, as appropriate (*e.g.*, should they be interested in additional addiction treatment, healthcare, or other services).

For study activities that involve contact with participants via telephone or video, study team members will only contact Veterans from a private area so no one outside of the study team can overhear the conversation or have access to research information or protected health information. The study team member that conducts a study activity via telephone or video will obtain the address of the Veteran's location where they will participate and the contact information of a person at or near the Veteran's location who can be called in the case of emergency. If there is not a person at or near the Veteran's location available in case of emergency, the study team member will notify the Veteran that they will call 911 if there is an emergency and provide the 911 dispatch with the Veteran's address. Veterans will participate via phone or video only if they have a safe, private location from which to participate. No phone or video contact will be recorded in any way.

Benefits

Subjects may benefit from HIV screens as well as medical laboratory tests. Those findings may be helpful in their clinical care if the patients wish the data to be shared with their clinicians. The study is not intended to directly benefit the subjects. As an indirect benefit to the subjects and others, the study may enhance collaboration between hepatology, substance use treatment, and mental health providers to bring about a higher level of coordinated patient care and substance use treatment. Collectively, these potential benefits outweigh the relatively small risks associated with filling out study questionnaires, submitting blood, saliva, and urine samples, and participating in neuroimaging assessments.

Protected Health Information

The date that biological specimens are collected, processed, and/or stored will be listed on the storage containers (*e.g.*, cryovials) utilized for this study. This identifier is used for quality control purposes in order to track specimens and monitor sample integrity/viability. The PHI to be collected is the minimum necessary needed to conduct the research and can't be further reduced.

The purpose of the data collected regarding drug abuse, alcoholism, alcohol abuse, and infection with HIV is to conduct scientific research, and no personnel involved in the study will identify, directly or indirectly, any individual patient or subject in any report of such research (*e.g.* manuscript or publication).

Multi-Site Study Concerns

Determining VA from Non-VA Research

This study will take place both at the Portland VA and at OHSU. VA Research will include consent, screening, breathalyzer and/or saliva alcohol testing, interviews, questionnaires, blood draws, oral fluid collection, urinalyses, and x-rays. Non-VA Research will include the MRI scans acquired at OHSU's Advanced Imaging Research Center (AIRC) Address: 3181 SW Sam Jackson Park Rd, L452, Portland, OR 97239; Phone: (503) 418-1505. In addition, some statistical analyses for this project will be performed by the Biostatistics and Design Program at OHSU. All data sent to OHSU for statistical analysis will be coded.

Costs To Subjects

None of the participants will pay for any of the procedures or visits in this study because they are only for research study purposes.

Subject Compensation

Subjects will be reimbursed $125 for the initial visits ($75 for visit 1 and $50 for visit 2). Subjects who participate in the 6-month follow up visits will receive an additional $125 ($75 for visit 3 and $50 for visit 4). In sum, subjects can earn $250 total if they complete all of the scheduled visits. Payment will be

provided in the form of electronic fund transfer (EFT) into the Veteran's bank account. This compensation is reasonable and commensurate with the expected contributions of the participant. The amount of payment and terms of the payment are described in the informed consent form. Payments are fair and appropriate and do not constitute (or appear to constitute) undue pressure or influence on, or coercion of, the prospective research subjects to volunteer for or continue participation in the research study.

Privacy and Confidentiality

All study data derived from subject procedures will be obtained specifically for research purposes. Research records will be maintained according to the VA research records retention schedule. Data will be stored in a manner intended to preserve patient confidentiality. Hard-copy protected health information (PHI) (*e.g.*, collected from clinical interviews and neuropsychiatric study visit procedures) will be stored in locked cabinets in the PIs VA office and laboratory space. Electronic PHI will be stored on servers behind the VA firewall accessed by password-protected VA computers. Each subject is assigned a unique identifier (study ID) based on a study identifier and number (*i.e.*, DAA-xxx). Only coded or de-identified data, not PHI, are used for analysis. The file linking the study ID to the patient's name will be stored in a separate password-protected file on a secure server behind the VA firewall. Coded data will be stored on OHSU's REDCap application, a highly secure and robust web-based research data collection and management system. No identifiable information will be entered into this application at any time. The statisticians will not have access to the code or PHI at any time. The spreadsheet linking the code to the subject will remain behind the VA firewall at VAPORHCS. Once the study has ended, the link will be stored by the VAPORHCS Research & Development Service.

DTI and MRI images will be collected at the AIRC at OHSU. No PHI is used to identify subjects and only the subject's coded ID is entered at the time of the scan. The raw data for each scan are downloaded to the Hoffman Lab (Co-I) Linux workstations in Building 100, room 5C-107 or Building 103, room E-141 at the VAPORHCS. Image analysis will take place on these workstations in VA space. The primary storage location for the coded neuroimages will be on a password-protected network drive at the OHSU Advanced Computing Center (ACC), to which only the research team has access. Study personnel will also analyze coded neuroimages using the ACC Exacloud System, a shared server which executes specific analysis scripts without removing data from its secure network drive.

Additionally, to complete image analysis remotely, study personnel will utilize an OHSU computer that is connected to the virtual private network (VPN) per OHSU ITG. This allows computers connected remotely to access the OHSU network and utilize network resources, including remote access of other network computers, while still having the same security level as being on campus. Using remote access software (TeamViewer, putty, etc.) for Linux workstations or MobaXterm software for accessing the ACC Exacloud System, study personnel will conduct image analyses remotely.

As participants are seen within clinical spaces for research visits, and to ensure that participants are in the right area for study visits, participants will check-in with front desk staff/MSAs of the appropriate clinic. Study staff will send appropriate MSAs an encrypted e-mail with participant name, date, and time of study visit to allow MSAs to perform normal clinical duties and assist participants in being routed to study staff.

Certificate of Confidentiality

To further protect subject's privacy and confidentiality, the investigators will obtain a Certificate of Confidentiality from the National Institute of Health. With this Certificate, the investigators cannot be forced (for example by court subpoena) to disclose information that may identify subject's in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings.

Information and/or Specimen Management

Blood specimens are collected from human subjects by study personnel or the VA phlebotomy lab specifically for research purposes. Specimens are coded with the subjects' unique identifiers and the date that the samples are processed or stored. The unique identifier matches the subjects' coded neuropsychiatric and demographic data. To track frozen sample storage, a hand-written log is maintained and secured in Dr. Loftis' laboratory. An electronic record is additionally created. Together with research staff, Dr. Loftis maintains viable samples by monitoring freezer temperatures and ventilation, tracking specimen identification and location, and training staff involved in any aspect of the process.

Clinical and laboratory data collected from consenting participants will be contributed into an existing IRB-approved VA Liver Disease repository (Lissi Hansen, PI, IRB #3891). Contribution to the repository is required, as stated in the informed consent form and the HIPAA authorization.

Transfer of Data Ownership

N/A

Data and Safety Monitoring Plan (DSMP)

Monitoring human participant recruitment, biological specimen collection, neuropsychiatric data collection, neuroimaging assessments, and data entry will be performed by the PI and study staff that performs the study visits and procedures. Any adverse events will be reported immediately to the PI and Dr. Chang (Co-I; Responsible Clinician) who will evaluate the patient and determine if any additional evaluation or treatment is needed. Dr. Loftis will ensure that adverse events are properly reported to the IRB. The study staff will examine all cumulative adverse events quarterly to determine if there are any systematic problems.

Study staff is trained on the protocols to ensure that procedures are being conducted within the scope of the approved protocols. Dr. Maya O'Neil, a licensed psychologist and neuropsychologist, trains and supervises the clinical research staff in the administration and scoring of neuropsychological tests and psychological measures. Dr. Hoffman, a psychiatrist and expert in neuroimaging, trains and supervises the research staff in neuroimaging procedures and data analysis. Recruitment and enrollment for clinical projects are reviewed regularly at Dr. Loftis' weekly laboratory meetings with research staff and at monthly meetings with Dr. Hoffman (Co-I). At these meetings, research staff provides enrollment updates, and the research team problem solves recruitment issues to ensure that projected enrollment numbers are obtained. The PI checks procedures (at least monthly) to verify that they are being conducted per the approved protocol, including the responsibilities and roles for gathering and monitoring data. The data are recorded by research staff as it is collected, and data accuracy is verified by double scoring, data entry and visual verification.

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Appendix – Supporting Documents List

Questionnaires and surveys

Alcohol Timeline Followback (TLFB) (Sobell, 1992, Sobell et al., 1996)
AUD Identification Test-C (AUDIT-C) (Bush et al., 1998)
Beck Depression Inventory, Second Edition (BDI) (Beck, 1996)
Brief Pain Inventory, Short Form (BPI) (Cleeland and Ryan, 1994)
Clinical Institute Withdrawal Assessment for Alcohol scale, revised (CIWA-Ar) (Sullivan et al., 1989)
Visual Analogue Scale (rates level of craving for alcohol)
Fatigue Severity Scale (FSS) (Krupp et al., 1989)
Generalized Anxiety Disorder Inventory (GADI) (Argyropoulos et al., 2007)
Neuropsychological Assessment Battery (NAB) (Stern and White, 2003)--attention, memory, and executive functioning modules
PCL-C (Weathers et al., 1994)
Reynolds Intellectual Screening Test (RIST) (Reynolds and Kamphaus, 2003).
Severity of Dependence Scale (SDS) (Gossop et al., 1997, Gossop et al., 1995)
Sickness Impact Profile (Bergner et al., 1981)
Structured Clinical Interview for DSM-5, research version (SCID-5-RV)
Wide Range Achievement Test, Fifth Edition (WRAT5™) (Wilkinson and Robertson, 2017)