Advancing Varenicline as a Treatment for Cannabis Use Disorder

A. SIGNIFICANCE

Overview of the Problem. The prevalence of cannabis use in the United States more than doubled between 2001 and 2013, from 4.1% to 9.5% of the adult population (Hasin et al., 2015). In 2014, over one million Americans received treatment for cannabis related problems (SAMHSA, 2015). Although a high demand for effective interventions exists, few specific treatments have been developed for cannabis use disorder (CUD). Further, current evidence-based treatments have limited efficacy, with few individuals achieving abstinence (Compton & Pringle, 2004; Kadden et al., 2007; Nordstrom & Levin, 2007; Vandrey & Haney, 2009; Sherman & McRae-Clark, in press). As such, it is critical to explore new strategies to improve treatment outcomes.

Nicotinic Acetylcholine Receptors as Potential Targets for CUD Medication Development. Nicotinic acetylcholine receptors (nACHr) are highly expressed in the mesocorticolimbic dopamine system (Dani & Bertrand, 2007) and also contribute to drug-related reward processes by impacting glutamate, and consequently dopamine, release (Fu et al., 2000; Kaiser & Wonnacott, 2000). As such, these receptors have been identified as potential targets for addiction treatment. Varenicline is a selective nACHr partial agonist of the α4β2 subtype and a full agonist of the α7 subtype (Mihalak et al., 2006), and is arguably the most effective first line pharmacotherapy for promoting tobacco cessation (Aubin et al., 2008; Eisenberg et al., 2008; Gonzales et al., 2006; Jorenby et al., 2006; Nides et al., 2006). Given its partial agonist profile, varenicline likely exerts its effects via dual mechanisms. First, it partially activates α4β2 receptors in the ventral tegmental area (VTA), resulting in increased dopamine levels and a reduction in withdrawal symptoms and craving (Rollema et al., 2007; Reperant et al., 2010). Further, through its antagonist properties, varenicline also blocks the ability of nicotine to further stimulate dopamine release, thereby attenuating nicotine's reinforcing effects during smoking (Coe et al., 2005). Varenicline reliably reduces reactivity to smoking-related cues among tobacco users via its effects on reward and cognitive circuitry (Brandon et al., 2011; Franklin et al., 2011; Hartwell et al., 2013).

Given that the mesolimbic dopamine system is a key element in the brain reward pathways and that increased dopaminergic transmission in these pathways is important for the reinforcing effects of multiple drugs of abuse (Roberts et al, 1980; Taylor & Robbins, 1984; Koob & LeMoal, 1997; Tanda et al, 1997; Volkow et al., 2016), varenicline has been identified as a prime candidate medication for evaluation in other substance use disorders (Crunelle et al., 2010). Positive findings have been reported in regards to varenicline reducing alcohol cue reactivity (Schacht et al., 2014), reducing alcohol self-administration among heavy drinking smokers (McKee et al., 2009), improving drinking outcomes in preliminary clinical trials (Fucito et al., 2011; Mitchell et al., 2012), and reducing alcohol use in a large, placebo-controlled trial (Litten et al., 2013). A recent case series also reported reductions in amount of enjoyment of cannabis and self-report of cannabis use among cannabis- and nicotine-dependent individuals receiving varenicline (Newcombe et al., 2015). To date, however, varenicline has not been evaluated in a controlled clinical trial for treatment of CUD.

Importantly, and particularly relevant to the present proposal, α4β2 nACHRs in corticothalamic circuitry, which are saturated with varenicline dosing (Lotfipour et al., 2012), have also been heavily implicated in prefrontally mediated attentional and inhibitory control (IC) (Sarter & Paolone, 2011). In addition, α7 nACHRs are involved in hippocampal-dependent memory function (Levin et al., 2006). nACHr agonists improve frontally mediated executive function among nicotine-naïve animals (Levin et al., 2006) and humans (Froeliger et al., 2009). Varenicline has been shown to improve multiple forms of attention (Rhodes et al., 2012) including inhibitory control (Austin et al., 2014) among treatment-seeking tobacco users and in nicotine-naïve animal models (Rollema et al., 2009; Terry et al., 2016). Given that cannabinoid agonists inhibit cholinergic transmission (Varvel et al, 2001; Lichtman et al., 2002; Vukadinovic et al., 2013), the cholinergic system in particular may play an important role in cannabis-induced cognitive dysfunction. As such, varenicline, as a cholinergic modulator in prefrontal circuitry, is a promising candidate treatment to ameliorate frontal-executive dysfunction (Sofuoglu et al., 2010).

Cognitive Function and Cannabis Use Outcomes. Cognitive impairments may predict poor response to behavioral treatments in drug users (Aharonovich et al., 2008; Caroll et al., 2011; Verdejo-Garcia et al., 2012), and pharmacotherapy targeting cognitive function has been proposed as a promising strategy for treatment of CUD (Sofuoglu et al., 2010). Acute and chronic effects of cannabis use on neuropsychological functioning have

been well characterized (for review, see Crean et al., 2011; Broyd et al., 2016; Curran et al., 2016). Laboratory experiments where controlled doses of cannabis have been administered reveal acute effects on attention processes (Morrison et al., 2009; Solowij et al., 1995). Similar memory impairments have also been demonstrated following chronic cannabis use (Solowij & Battisti, 2008). Of note, greater cognitive deficits are associated with number of lifetime cannabis use episodes (Medina et al., 2007) and with severity of CUD (Filbey & Yezhuvath, 2013).

Inhibitory Controland Varenicline. Inhibitory control (IC) tasks have proven to be an effective probe of executive function in individuals with a substance use disorder (Moeller et al., 2016). Cannabis users (Tapert et al., 2007) and nicotine dependent smokers (Froeliger et al., 2012; Froeliger et al., 2013; Kozink et al., 2010) demonstrate a pattern of dysregulated hyperactivity in lateral prefrontal (i.e. right inferior frontal gyrus: R. IFG) BOLD response during attention and inhibitory control tasks, without benefits in task performance, suggestive of a compensatory mechanism to perform task demands. This is significant, as the R.IFG is a key node in a corticothalamic circuitry that mediates inhibiting a prepotent motor response in order to execute a goal directed behavior (Rae et al., 2015; Swann et al., 2012). Moreover, hyperactivity in IFG-BOLD response during inhibitory control tasks is associated with worse cessation outcomes across multiple substance use disorders (see Moeller et al., 2016). When taken together, those findings are consistent with our pilot data from smokers (*N*=26) performing an IC task which reveals that less task-related IFG BOLD response and stronger IC task-based functional connectivity between R.IFG and thalamus (henceforth corticothalamic circuit) is associated with: forgoing smoking for a longer period of time, and upon initiation, lighter smoking during ad lib smoking in the lab (see Preliminary Data). Work in an α7 knockout mouse model implicates an important role of the α7 receptor in mediating IC (Hoyle et al., 2006), and in tobacco users varenicline improves aspects of inhibitory control (Austin et al., 2014), posited as a key mechanistic aspect of varenicline's established efficacy for tobacco cessation. Varenicline improves attentional control among healthy non-smokers (Mocking et al., 2013) and smokers in a state of nicotine withdrawal (Patterson et al., 2009). In summary, the extant literature in animal and human models of substance use disorders, including CUD, reliably demonstrates that deficits in executive functionthat are associated with the ongoing maintenance of drug use may be amenable to treatment with varenicline.

Comorbidity of Cannabis and Tobacco Use Disorders. Cannabis and tobacco use often co-occur (SAMHSA, 2015), and approximately half of adults seeking treatment for cannabis use also use tobacco (Peters et al., 2012). Individuals using both cannabis and tobacco have been shown to have more psychosocial problems (Moore & Budney, 2001) and worse cannabis use treatment outcomes (de Dios et al., 2009; Gray et al., 2011; Moore & Budney, 2001) than individuals using cannabis alone. However, to date, limited research has focused on interventions targeting both cannabis and tobacco use. Given its proven efficacy in tobacco cessation, it is possible that varenicline may improve both cannabis and tobacco use outcomes in co-using individuals. Of note, though, in the work of Litten and colleagues (2013), the impact of varenicline on drinking was similar for smokers and nonsmokers, suggesting that varenicline's efficacy in this population was independent of its tobacco cessation effects.

Adherence and Varenicline Treatment Outcomes. An evaluation of varenicline adherence found that of 1,477 patients prescribed varenicline for smoking cessation, only 24% were adherent to the three-month course of therapy (Liberman et al., 2013). Importantly, adherent individuals were 93% more likely to quit smoking than non-adherent or partially adherent individuals. Similarly, Catz and colleagues (2011) found in the COMPASS trial that good adherence to varenicline was associated with a two-fold increase in 6-month smoking quit rates compared with poor adherence (52% vs. 25%). An analysis of factors that influenced adherence in the COMPASS trial found the most frequently endorsed reasons for premature medication discontinuation were side effects and perceived lack of need (Catz et al., 2011). These findings suggest that adherence interventions providing timely side effect management and encouraging longer term use of medications are needed to maximize treatment effectiveness.

Summary and Scientific Premise. There is a critical need to develop safe and effective medications for CUD. Impairment in executive function is observed across substance use disorders and is particularly relevant to CUD (Cabrera et al., 2016; Curran et al., 2016). Inhibitory control, a clinically important and measurable component of executive function, is (a) impaired in CUD (Filbey & Yezhuvath, 2013; Nicholls et al., 2015; Tapert et al., 2007), (b) reliably measured by our team (Froeliger et al., 2012, 2013; Preliminary Data), (c) predictive of substance

use disorder cessation outcomes (Moeller et al., 2016), and (d) a known key component of varenicline's mechanism (Austin et al., 2014; Lotfipour et al., 2012). Varenicline's application to individuals with CUD represents a mechanistically intriguing avenue of research, with potential promise of improving clinical outcomes. We thus propose an initial UG3 proof-of-concept trial focused on functional imaging to evaluate mechanism as well as to collect preliminary clinical outcome data on varenicline's impact of cannabis-related withdrawal and cannabis use. If warranted by positive UG3 findings, a subsequent fully-powered UH3 trial will comprehensively evaluate safety and efficacy. The UG3/UH3 mechanism is ideally suited to efficiently yet thoroughly evaluate a medication with strong rationale but no prior trials within the target population.

Even if the UG3 "proceed-or-not-proceed" hypothesis is refuted, the UG3 would cost- and time-effectively provide a wealth of functional imaging and behavioral data important to broadening our understanding of CUD and potential pathways for pharmacological treatment. If, instead, the UG3 hypothesis is supported, we would be able to efficiently proceed with a fully powered trial to comprehensively evaluate varenicline's safety and efficacy in CUD.

B. INNOVATION

The proposed research is innovatively designed to efficiently and strategically evaluate varenicline as a promising candidate pharmacotherapy for CUD. The UG3 proof-of-concept trial incorporates advanced functional neuroimaging to evaluate varenicline's effects on inhibitory control and cannabis cue reactivity, with clear criteria to determine whether or not to proceed with the fully-powered UH3 clinical trial to comprehensively evaluate varenicline's safety and efficacy for CUD. This innovative approach is consistent with the field's emerging use of neuroimaging to assess the effectiveness of substance use disorder treatments (Cabrera et al., 2016). In both trials, an advanced medication adherence system will be utilized to both accurately measure adherence as well as enhance medication taking behavior throughout the trial.

C. RESEARCH DESIGN

Capacity of Research Team. Completion of the proposed research will require experience with recruitment and retention of individuals with CUD, varenicline clinical trial management, administration of neurocognitive tasks and cannabis cue reactivity within functional magnetic resonance imaging (fMRI) paradigms, and adherence monitoring. As detailed below, the research team has a proven track record in these research areas. The proposed project will be co-directed by Drs. Aimee McRae-Clark and Kevin Gray, productive clinical researchers with NIH-funding primarily focused on clinical trials and human laboratory work with cannabis and tobacco using individuals. Co-investigators will also contribute meaningfully to the conceptualization, conduct, and reporting of this research. Dr. Brett Froeliger leads multiple NIH-funded projects utilizing fMRI techniques to characterize the impact of drug use on neurocognition and to predict clinical outcomes. Dr. Lindsay Squeglia has expertise in cannabis cue reactivity paradigm development and fMRI modeling of cue induced craving.

Experience with Recruitment and Retention of Individuals with CUD. Dr. McRae-Clark has completed multiple NIH-funded studies involving cannabis using individuals (McRae-Clark et al, 2009; McRae-Clark et al, 2010; McRae-Clark et al. 2015; McRae-Clark et al., 2016). Dr. Gray also has led multiple trials assessing medications in individuals with CUD, including the recently completed NIDA Clinical Trials Network Achieving Cannabis Cessation—Evaluating N-Acetylcysteine as a Treatment (ACCENT) protocol (Gray et al., 2012; McClure et al., 2014). We have an active recruitment network in place, and have been able to consistently surpass recruitment goals.

Varenicline Clinical Trial Management. Drs. McRae-Clark and Gray have significant experience in the management of individuals receiving varenicline, both clinically and in the context of research protocols (Gray et al., 2012; Gray et al., 2015; Hartwell et al., 2013; McClure et al., 2015). Dr. Gray is currently conducting a clinical trial assessing the safety and efficacy of varenicline in an adolescent population (U01DA031779; PI: Gray) as well as a trial examining the combination of varenicline and N-acetylcysteine for smoking cessation (R01DA038700; PIs: Froeliger, Gray, and Kalivas).

Neurocognitive Tasks. In an ongoing laboratory study (R01DA033459), Drs. Froeliger and Gray are examining relations between corticothalamic neural circuitry mediating IC (Rae et al., 2015) and the ability to refrain from smoking behavior during a laboratory-based smoking resistance task (SRT). Sated smokers (n=26) were first fMRI scanned while performing the GoGo/NoGo task that is proposed herein (Chikazoe et al., 2009), a well-

validated probe of IC and a process posited to be a transdiagnostic factor of relapse vulnerability (Moeller et al., 2016). Immediately following the fMRI phase, smokers performed the SRT in which a monetary contingency is in place to refrain from smoking while being presented with smoking cues in the laboratory for up to one hour. When smokers elected to smoke in favor of earning money to remain abstinent, smoking topography (e.g., # of puffs) was recorded and relations with fMRI BOLD signal examined. We found that worse task performance (Fig 1, Box A: R=.41, *p*=.04) and exaggerated BOLD response in R. IFG (Fig 1, Box B: t= 3.54, p<.05) predicted smoking sooner during the SRT; weaker task-based functional connectivity in corticothalamic circuitry (Fig 2, Box C) predicted smoking more heavily upon initiation. These findings are consistent with the literature demonstrating that dysregulated prefrontal function during IC is predictive of worse treatment outcomes (Moeller et al., 2016), yet also extends the literature by linking the corticothalamic pathway (Jahanshahi et al., 2015) to the ability to resist drug use.

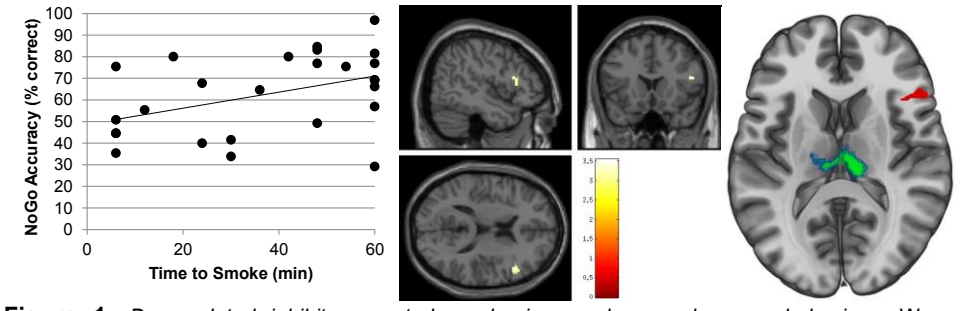
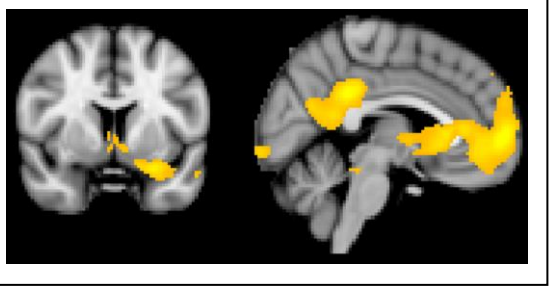


Figure 1. *Dysregulated inhibitory control mechanisms subserve drug-use behavior.* Worse task performance (Fig x, Box A: β = 0.08, R^2 = .20) and exaggerated BOLD response in right IFG (Fig x, BoxB: β= -1.7, R^2 = .35) predict smoking sooner during the SRT. Using the functional defined rIFG (Box B) as a seed (Box C-red cluster) in a task-based functional connectivity (tbFC) analysis, weaker connectivity with the thalamus (Box C-green cluster: -corticothalamic circuit) predicted smoking more heavily upon initiation; including # puffs (r=-.44, p=.02), total puff volume (r=-.51, p=.008) and total puff duration (r=-.46, p=.018). Significance for *fMRI statistics was defined at α = .05 (Monte Carlo: p<.005;).*

Cannabis Cue Reactivity (CR). Drs. McRae-Clark and Gray have developed CR paradigms and demonstrated an ability to elicit cannabis craving in both adult (McRae-Clark et al., 2011) and adolescent (Gray et al., 2008) populations, and have experience with fMRI evaluation of varenicline's effects on tobacco cue reactivity (Hartwell et al., 2013). Recently, Drs. Squeglia and Gray have developed a cannabis fMRI CR task, with cues relevant to US populations, to investigate cue-elicited activation. Cannabis and neutral images were matched on factors such as color, complexity, and context. A mix of active (e.g., smoking a joint) and passive (e.g., cannabis plant) pictures were used. Data were collected in a pilot study of 25 regular, heavy cannabis users (48% women, mean age = 18.7 ± 0.51; using cannabis twice daily on average). Analysis of the fMRI data showed significant activation (voxel-level z=3.7, *p*=.0001; cluster-corrected *p*<.05) in bilateral medial prefrontal, striatum, anterior cingulate, subcallosal, precuneus, and posterior cingulate cortex during the cannabis vs. non-cannabis neutral cues (see Figure 2). These results are consistent with those found in a Dutch-specific cannabis CR task (Cousijn et al., 2013) and a tactile cannabis task (Filbey et al., 2009), suggesting the cannabis cues developed for this task were effective at eliciting increased activation in brain regions involved in reward processing. Advantageously, this task displays cues relevant to American cannabis users, covers a range of cannabis use methods, and does not require a tactile component.

Figure 2. Robust activation was elicited from cannabis, compared to neutral, stimuli in heavy cannabis users in reward regions including the medial prefrontal cortex and anterior cingulate highlighted below (n=25).

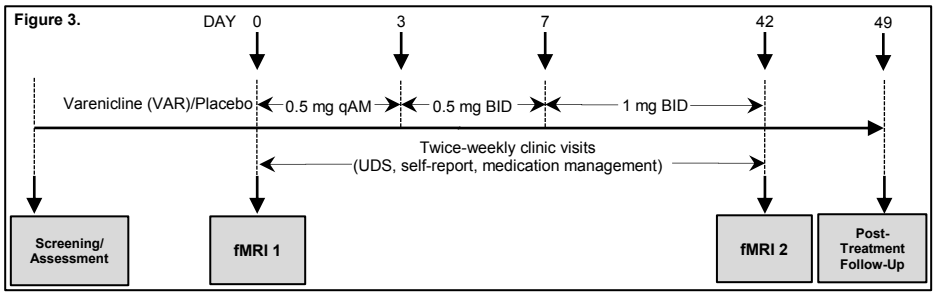


Adherence Monitoring. All varenicline and placebo tablets will be dispensed in bottles equipped with a MEMS 6 TrackCap. In addition, subjects will record themselves taking their morning and evening medication doses and then submit these videos to research staff via RedCap survey. Subjects may use their personal smartphones for video submission. If they do not have a smartphone, one will be loaned to them during the course of the study, as video submissions may only be completed on a smartphone (cannot be completed on a computer). A survey link will be sent to the subject via text message twice daily. Video capture will occur as part of the RedCap

survey. Videos are automatically stored on some Android smartphones, and participants will be informed of that so they can delete the files, if necessary. Participants using iPhones (and using loaner iPhones from our group) will not have stored videos on their phone and nothing will need to be deleted.

Research Design and Methods. The first phase (UG3) is a proof-of-concept pilot study to test mechanistic hypotheses and preliminarily evaluate safety and initial efficacy of varenicline in cannabis using individuals. Clearly defined milestones, focused on varenicline versus placebo reduction in cannabis related outcomes, will be met before proceeding to Phase 2 (UH3), a fully powered clinical trial to assess clinical outcomes to potentially advance the FDA approval of varenicline for the treatment of CUD.

UG3 Overview. As shown in Figure 3, if there is scanner availability within approximately 2 weeks, fMRI-eligible individuals with CUD will complete an initial fMRI paradigm including GoGo/No-Go inhibitory control and cannabis cue reactivity tasks. Subjects who are not fMRI eligible or are unable to be scheduled for scanning within 2 weeks of screening will complete these same behavioral scanner tasks via computer in the clinic (pre- and post-varenicline versus placebo treatment). All participants will be randomized to a proof-of-concept six-week course of varenicline or placebo treatment, an abbreviated course chosen to efficiently detect mechanistic and preliminary clinical signals of varenicline's effects (Gray et al., 2015; Saladin et al., 2015). Cannabis use outcomes (urine cannabinoid tests and self-report) will be measured at twice weekly clinic visits, and at a post-treatment follow-up safety visit. A repeat fMRI session will be completed with eligible individuals who completed an initial scan at the end of the 6-week treatment period to evaluate varenicline versus placebo differences in inhibitory controland cannabis cue reactivity.



Participants. A total of 72 participants with CUD, aged 18 and over and using cannabis at least 3 days per week, will be recruited over an 18-month period. Additional inclusion criteria include consent to random assignment, ability to read and provide informed consent, and interest in CUD treatment. Exclusion criteria include women who are pregnant, nursing, or planning to become pregnant during the course of the study; having a history of bipolar, psychotic or medical disorder that would limit ability to participate; and meeting criteria for any moderate or severe non-cannabis substance use disorder. Detailed inclusion/exclusion criteria are in Human Subjects.

Recruitment. Recruitment is planned to occur through both clinical referral and advertising. This approach has been used effectively for years in large-scale CUD clinical trials at MUSC.

Procedures.

Strategies to Ensure a Robust and Unbiased Approach. As detailed throughout this section, the proposed study will achieve robust and unbiased results via several design features including: explicit inclusion/exclusion criteria; randomization of treatment condition; placebo control; blinding; use of validated measures and methods; explicit hypotheses and corresponding planned statistical analyses; power estimates; planned handling of retention/attrition and missing data; objective adherence monitoring; and careful consideration of potential confounds.

Screening and Eligibility Assessment. Individuals will be screened by the research study intake coordinator. An initial pre-screen, focused on inclusion/exclusion psychiatric diagnoses, medical status, current medication regimen, and ability and willingness to commit to completion of study procedures, will be used to initially determine potential study eligibility. Interested individuals will be given a full description of the study procedures and asked to read and sign an IRB-approved informed consent form before participating in a detailed, comprehensive screening and assessment phase.

Diagnostic/Descriptive Assessment. The MINI International Neuropsychiatric Interview (MINI) will be used to assess psychiatric and substance use diagnoses. A medical history, physical exam, laboratory assessment (comprehensive metabolic panel and complete blood count) will be completed. An fMRI safety screening

questionnaire will be conducted to determine if the individual is eligible to undergo the fMRI scanning portion of the study. In the event that an individual is found to be ineligible to participate in this research protocol completely, he or she will be given an appropriate referral for further medical care or to an appropriate treatment program. If found eligible, a randomization visit, including fMRI scanning session if eligible, will be scheduled, and ongoing cannabis use will be tracked between initial assessment and randomization.

Treatment Assignment. Eligible individuals will be randomized to receive double-blind varenicline or matching placebo. Randomization (stratified on gender and smoking status) and dispensing will be performed by the MUSC Investigational Drug Service, a centralized research pharmacy that compounds and manages clinical trial medications. Matching varenicline and placebo tablets will be provided by Pfizer, at the standard recommended dose of 0.5mg daily for three days, then 0.5mg twice daily for four days, and then 1mg twice daily for the remainder of the six-week treatment period. If necessary, medication dose may be reduced to 0.5mg twice daily for tolerability. Medication treatment will be initiated following completion of the fMRI paradigm and/or baseline visit.

Scan sessions. If there is scanner availability within approximately 2 weeks, participants who qualify for fMRI procedures will undergo two fMRI scans (pre- and post-varenicline versus placebo treatment). In order to facilitate ease of scanner scheduling, subjects' scanning and initial therapy session visit may be conducted on separate days if needed. Subjects will be instructed to abstain from cannabis and alcohol for a minimum of 12 hours prior to scanning to avoid acute intoxication during procedures. Breath (to determine carbon monoxide and alcohol levels) and urine toxicology samples will be collected before each scan. In addition, participants will also be asked to provide a saliva sample to verify abstinence from recent cannabis use through use of SalivaConfirm® testing (Confirm Biosciences, Inc.). Pre- and post-fMRI state craving measures will be collected. Each imaging session will include a resting-state, inhibitory control, and cannabis cue reactivity task scan and last approximately 60 minutes total.

T1-weighted structural: A high-resolution anatomical scan (magnetization prepared rapid gradient echo) will be acquired to allow subsequent registration to functional images and region-of-interest (ROI) definition (parameters: repetition/echo time (TR/TE)= 1900/2.26 ms; flip angle (FA)= 9°; field of view (FOV)= 256 mm^2; voxel size= 1 mm^2; 192 contiguous 1-mm-thick slices).

Resting–state functional connectivity (rsFC): The resting-state scan consists of a 6-minute, eyes-closed period. Similar to our previous work (Froeliger et al., 2015), rsFC will be assessed using the conn13 SPM8 toolbox. First, experimental design variables, pre-processed functional images will be filtered with a 0.01 to 0.08 Hz band-pass filter and normalized (modulated to preserve volume). 5-mm spheres will be created around MNI coordinates for a priori regions of interest (ROIs) that include: insula, nucleus accumbens, dACC, rACC and inferior, middle and superior frontal gyri. The conn13 toolbox uses PCA to isolate potentially confounding noise from nuisance covariates using default settings. Individuals' white matter and CSF templates, in-scanner heart-rate (HR), respiration rate and movement parameters will be included as covariates. Connectivity matrix for each seed will be entered into separate 2 (Varenicline: Yes, No) x 2 (Time: Scan1, Scan2) ANOVA models. We will follow-up with exploratory whole brain analyses.

GoGo/NoGo Inhibitory Control (IC) Task. During the inhibitory control task, which will be the main task of interest in determining whether or not to proceed from the UG3 mechanistic proof-of-concept project to the fully-powered UH3 clinical trial, fMRI BOLD response will be collected from participants as they perform a "GoGo/NoGo" task (Chikazoe et al., 2009). Participants are instructed to press a button in response to common (75% of trials) and rare (12.5%) Go stimuli while inhibiting responding to rare NoGo stimuli (12.5%). The task provides errors of omission and reaction times during Go trials, and errors of commission on NoGo trials. *Behavioral performance* data will be analyzed in SAS. *fMRI task* data will be entered into a first-level, whole-brain analysis using the General Linear Model to examine BOLD response to each of the 5 trials of interest: $NoGo_{correct}$, $NoGo_{incorrect}$, $RareGo_{correct}$, $RareGo_{incorrect}$, and $Go_{incorrect}$. Each event will be modeled as a delta regressor (onset dur. = 0) and convolved with a canonical hemodynamic response function. Motion will be removed through rigid body rotation and translation and parameters included as covariates. A high-pass filter (128 seconds; .008 Hz) will be applied to remove slow signal drift.

The Cannabis Cue Reactivity (CR) Task. During the cannabis cue reactivity task, participants are shown pseudorandomly interspersed images of cannabis (i.e., cannabis plant, cannabis-related paraphernalia) and neutral (e.g., pine cone, trumpet) images, visual control images (i.e., blurred images), and a fixation cross (see Figure 4). The cannabis stimuli were matched by color, hue, and complexity. Blurred images and the fixation crossed trials are used as contrasts to evaluate attention and non-cannabis specific effects. Based on pilot data (see above), this task reliably activates reward regions, including bilateral medial prefrontal, striatum, anterior cingulate, subcallosal, precuneus, and posterior cingulate cortex in heavy cannabis-users. Stimuli are presented in six 120-s epochs, each consisting of four 24-s blocks of an image type (one block each of cannabis, non-cannabis control, and fixation). Each block is followed by a 6-s washout period, allowing the hemodynamic response from the previous block to decline before the next is presented. A 12-m gradient-echo EPI sequence will be acquired (parameters: repetition/echo time (TR/TE)= 2200/35 ms; flip angle (FA)= 90°; field of view (FOV)= 220 x 220 mm; voxel size= 3.00 x 3.00 mm; 37 contiguous 3-mm-thick slices). A magnetic **fieldmap** will also be acquired to allow geometric unwarping and cost-function masking of EPI images induced by magnetic field inhomogeneities.

Figure 4. Cannabis cue reactivity paradigm with active and passive cues. BOLD response during cannabis (e.g., cannabis plant, joint) vs. neutral (e.g., pine cone, trumpet) cues will be the main contrast of interest.

Psychosocial Treatment. All participants will receive brief motivational enhancement therapy consisting of three individual sessions. The first session will occur during the first week of medication administration, and the second session will occur approximately one week later. Sessions will incorporate use of a personalized feedback report summarizing the participant's problems related to use, reasons for quitting, and high-risk situations for use. The major goals of the first session will be to build rapport, identify issues related to health behavior change, and goal setting. The second session will focus on assessment/review of goals and barriers to goal achievement. The third session will occur at approximately Week 4 and will be used to follow-up on action plans. We have successfully used a similar intervention in previous cannabis treatment studies (McRae-Clark et al, 2009; McRae-Clark et al, 2010; McRae-Clark et al. 2015) to provide an evidence-based treatment platform for all participants.

Assessments. **Table 1** provides an overview of assessments with a brief description of the instruments below.

	SCR	fMRI1/ RAN	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6/ fMRI2	FU
Pre-screen, MINI, H&P, CBC/CMP	×								
Self-Efficacy, Reasons for Quitting	×								
Marijuana Problem Scale, CGI-S	×							×	×
Urine Pregnancy Test	×	×				×		×	
TLFB, HAM-A, HAM-D, C-SSRS, MCQ, CWS, PSQI	×	×	×	×	×	×	×	×	×
Barratt Impulsiveness Scale	×							×	
Urine Drug Test, Carbon Monoxide & Alcohol Breathalyzers	×	×	××	××	××	××	××	××	×
Saliva Drug Test		×						×	
Urine Cotinine	×		×	×	×	×	×	×	×
Adverse Events, ConMeds, Medication Management	×	×	×	×	×	×	×	×	×
RedCap, Pill Count			×	×	×	×	×	×	
SCR=Screen, RAN=Randomization, FU=Follow-Up									

1. Screening and Diagnostic Instruments. **Pre-Screen:** This assessment will be used to determine whether an individual is likely to meet inclusion or exclusion criteria for the study when they first present. The instrument is designed to assess for substance use disorders and obvious psychiatric, medical, and logistic exclusions. **Mini-International Neuropsychiatric Interview (MINI):** The MINI is a brief structured interview that was designed to assess DSM-5 diagnoses using a series of questions in dichotomous format (yes/no) (Sheehan & Lecrubier,

2003; Sheehan et al, 1998).

2. Psychiatric and Functioning Assessments. **Clinical Global Impression of Severity Scale (CGI-S):** The CGI-S (Guy, 1976) is used to record the severity of illness at time of assessment on a scale of 1 (normal, no illness) to 7 (among the most extremely ill patients). **Hamilton Anxiety and Depression Rating Scales (HAM-A & HAM-D)**: These validated scales assess severity of anxiety and depressive symptoms (Hamilton, 1959, 1960). **Barratt Impulsiveness Scale (BIS-11)**: This questionnaire is designed to assess the personality/behavioral construct of impulsiveness (BIS-11; Patton et al., 1995). **Pittsburgh Sleep Quality Index (PSQI):** The PSQI is a standardized measure of sleep quality that assesses a variety of sleep disturbances over the past week or month (Buysse et al., 1989). **Columbia-Suicide Severity Rating Scale (C-SSRS):** The C-SSRS (Posner et al., 2011) is a brief, low-burden suicide assessment scale administered by a clinician. We have successfully used this instrument in multiple previous trials, and it is regarded as the gold standard of suicidality assessment in clinical trials.

3. Substance-Related Instruments. **Time-Line Follow-Back (TLFB):** The TLFB (Sobell & Sobell, 1992) is a calendar-based instrument designed to assess daily substance consumption. Although initially designed to assess alcohol use, we have successfully modified this instrument to assess for other drug use. Cannabis use will be recorded as times used per day, with each time being defined as cannabis use separated by an hour of no cannabis. We will use established methods to standardize for different types of cannabis use (joints, bowls, blunts, etc.), as well as determining overall amount used per day. Tobacco, alcohol, and other substance use will also be assessed. **Urine Drug Testing:** The urine drug tests will qualitatively screen for the presence of opioids, cocaine, amphetamines, and benzodiazepines. Urine cannabinoid tests will be performed using the AXSSYM® system from Abbott Laboratories. This assay is semi-quantitative with a detection cut-off value of 30.00 ng/ml (Abbott AXSSYM® System package insert). Urine creatinine will also be obtained, as creatinine normalization has been proposed as a method to differentiate new cannabis use from residual drug excretion (Huestis and Cone, 1998; Schwilke et al, 2011). **Saliva Drug Testing:** In addition to urine testing, participants will also be asked to provide a saliva sample prior to scanning sessions to verify recent abstinence from cannabis use through use of SalivaConfirm® testing (Confirm Bioscience, Inc.). This test is able to detect THC in saliva for up to 14 hours. **Carbon Monoxide Breathalzyer:** This method will be used during all study visits to detect residual levels of carbon monoxide from recent smoked tobacco or cannabis use. A “cut-off” of 8 parts per million will be used as a biological abstinence confirmation measure (SRNT Subcommittee on Biochemical Verification, 2002). **Urine Cotinine:** Nicotine is metabolized to cotinine by the liver. Cotinine has a longer half-life than nicotine, and thus serves as a more reliable biomarker of cigarette smoking (Zevin et al., 2000). Consensus guidelines support urine cotinine testing to biologically confirm 7-day abstinence, with a recommended “cut-off” of 50ng/ml (SRNT Subcommittee on Biochemical Verification, 2002). In the UG3 trial urine cotinine will be measured at screening as biological confirmation of smoking status. Urine cotinine measurement will be repeated weekly for those who are determined to be smokers. **Marijuana Craving Questionnaire (MCQ):** The MCQ (Heishman et al, 2001) is a Likert-based self-assessment of cannabis craving shown to be a valid and reliable instrument for measuring cannabis craving. The 12-item MCQ will be used, which has been constructed by selecting the three items from each factor of the full 47-item MCQ that exhibited the most within-factor reliability (Heishman et al, 2009). **Cannabis Withdrawal Scale (CWS):** The Cannabis Withdrawal Scale (Allsop et al, 2011) is comprised of 19 items for which participants rate severity of symptoms in the previous 24 hours. **Marijuana Use Summary Sheet/Self-Efficacy Questionnaire/Marijuana Problem Scale/Reasons for Quitting Questionnaire:** These worksheets, created by Stephens and colleagues (2000), will be used to gather information from participants to prepare personalized feedback reports for use in the motivational enhancement sessions.

4. Safety Assessment. **Medication Management, Concomitant Medications, and Adverse Event Evaluation:** Using our team’s established procedures, medication management, including tracking of concomitant medications, review and encouragement of cannabis abstinence and study medication adherence, and adverse event evaluation, will be led by the medical clinician. Adverse events (AEs) will be assessed serially. The type of AE, severity of AE, and the relationship to study medication will be recorded. AEs will be coded on a weekly basis using Medical Dictionary for Regulatory Activities (MedDRA) rules.

5. Adherence. Pill counts will be completed at each clinic visit. Adherence will also be monitored using MEMS 6

Trackcaps. In addition, as outlined previously, subjects will be asked to record and upload videos of themselves taking each medication dose via smartphone and RedCap.

Follow-up plan. Following the 6-week trial, a post-treatment follow-up visit will occur, and participants will be referred for appropriate substance use management.. In the event that an AE occurs during the study, a participant will be followed until resolution.

Primary and Secondary Outcome Measures

Outcomes will be compared between participants randomized to receive double-blind varenicline versus placebo.

The primary efficacy outcome will be cannabis withdrawal during active treatment, as measured by the negative affect items of the Cannabis Withdrawal Scale (items 5 [“I felt nervous], 6 [“I had some angry outbursts”], 7 [“I had mood swings”], 8 [“I felt depressed”], 9 [“I was easily irritated”], 15 [“Life seemed an uphill struggle”], 18 [“I felt physically tense”], restlessness (item 11, “I felt restless), and/or urge to smoke (items 1 and 10, “The only thing I could think about was smoking some cannabis” and “I had been imagining being stoned”). These items were chosen based on previous trials of varenicline in tobacco smoking cessation trials (Jorenby et al., 2006; Gonzales et al., 2006).

Secondary efficacy outcomes will include a) cannabis use quantity (reduction between baseline and end of treatment); b) cannabis abstinence (weeks 3-6 of active treatment, after the initial 2-week grace period, inclusive of medication titration and initial targeted quit date); and c) cannabis craving as measured by the Marijuana Craving Questionnaire during active treatment. Cannabis abstinence will be defined based on values published by Schwilke et al. (2010) in which residual urinary excretion was modeled in chronic cannabis users, with gender and racial corrections for urinary creatinine excretion (Barr et al., 2005). As such, the criterion for abstinence will be set at 300 ng/mg CN-THCCOOH for African American male participants, 350 ng/mg CN-THCCOOH for non-African American male participants and African American female participants, and 500 ng/mg for non-African American female participants. In exploratory fashion, we will additionally examine mood (Hamilton Depression Rating Scale [HAM-D]), anxiety (Hamilton Anxiety Rating Scale [HAM-A]), concentration (HAM-A item 5 [difficulty in concentration]) and sleep (Cannabis Withdrawal Scale items 12 [“I woke up early”], 14 [“I had nightmares and/or strange dreams”], and 17 [“I had trouble getting to sleep at night”]; HAM-D items 4-6 [initial, middle, and delayed insomnia]; HAM-A item 4 [insomnia]; and Pittsburgh Sleep Quality Index).

Secondary safety outcomes will be a) the frequency of treatment-emergent adverse events (AEs), an approach similar to those of varenicline phase III smoking cessation studies (Gonzales et al., 2006; Jorenby et al., 2006); b) treatment-emergent adverse events leading to medication discontinuation and c) the occurrence of treatment-related serious adverse events (SAEs). Adverse events will be assessed by trained clinicians with significant experience in varenicline clinical trial conduct. All AEs will be evaluated for potential causality to study drug treatment. Depression and anxiety will be reflected by HAM-D and HAM-A scales ratings, suicidality will be reflected via C-SSRS ratings.

The exploratory mechanistic outcomes will include a) corticothalamic pathway BOLD response signal change from baseline during the fMRI inhibitory control Go-No Go task, b) corticolimbic negative affect pathway BOLD response signal change from baseline during the fMRI negative emotional cue reactivity task, c) mesocorticolimbic reward pathway BOLD response signal change from baseline during the fMRI cannabis cue reactivity task, and d) strength of resting state functional connectivity in relation to phenotypic differences (e.g., impulsivity as measured by the Barratt Impulsivity Scale, cannabis use disorder severity as measured by DSM-5 criteria met for CUD by the MINI diagnostic interview, and trait-negative affect as measured by the HAM-A and HAM-D).

Statistical Analysis

Data Management Plan. All data will be entered into a standard software package. Macro programs will be written to check the data for logical consistency and values out of possible range. Quarterly database management and data integrity audits will be conducted.

Efficacy: Primary Analysis
Categorical clinical and demographic variables will be assessed by chi-square tests of independence, while continuous variables will be assessed using Student's t-test. In addition to baseline group differences, preliminary analysis of baseline characteristics with negative affect/withdrawal and cannabis use outcomes of interest will examine significant correlates of abstinence functional deficits. Characteristics significantly associated with these outcomes will be included as covariates in the initial stages of model development. Cannabis specific withdrawal and negative affect (Hamilton Anxiety Rating Scale [HAM-A], Hamilton Depression Rating Scale [HAM-D], negative affect component of Cannabis Withdrawal Scale [CWS]) will be measured at weekly visits during the treatment portion of the study. General linear mixed effects models will be developed to test the efficacy of treatment with varenicline as compared to placebo in reduction of measured withdrawal and negative affect during weeks 3-6. Model assumptions will be verified using analysis of residual and appropriate transformations will be employed when necessary.

Safety: Primary Analysis
Similar to varenicline phase III tobacco cessation studies (Gonzales et al., 2006; Jorenby et al., 2006), we define a treatment-emergent adverse event as any adverse event occurring between treatment initiation and one week following treatment conclusion. Non-inferiority analysis will be utilized to compare adverse event rates between varenicline and placebo groups (Piaggio et al., 2006).

Secondary Outcomes Analyses
Secondarily, we plan to preliminarily investigate the efficacy of varenicline, compared to placebo, in reducing cannabis use quantity (Timeline Follow-Back) and increasing the proportion of cannabis-abstinent participants (urine cannabinoid test, Weeks 3-6; abstinent definition provided above). A combination of self-reported cannabis use as well as negative urine cannabinoid tests (UCTs) during Weeks 3 through 6 will be sufficient to estimate cannabis use quantity changes and assign abstinence (yes/no) and assess the treatment effect on clinical outcomes. General linear mixed effects models will be used to estimate changes in cannabis use and differential effects of treatment on the use patterns. Logistic regression models will be used to assess continuous abstinence proportions across treatment assignments. Analysis models will be reported both unadjusted and adjusted for significant clinical covariates (determined as associated with abstinence from the baseline analysis as well as known clinical confounders). Additionally, we plan to analyze the effect of varenicline on weekly abstinence from cannabis across the full treatment course. Generalized linear mixed effects models will be constructed to estimate treatment group differences in abstinence across the entire time course using the methods of generalized estimating equations (GEE). Additionally, data on cannabis craving (MCQ) will be collected from all participants at weekly visits. Generalized linear mixed effects models will be constructed to estimate treatment group differences in craving scores during the treatment phase of the study.

To evaluate the impact of varenicline on inhibitory control, hypothesis testing will be conducted by (a) entering NoGocorrect -RareGocorrect contrast images into a 2 (Group: varenicline, placebo) x 2 (Time: Pre, Post) rmANOVA and examining within an IC mask that includes right inferior frontal gyrus, preSMA, thalamus and primary motor cortex, and (b) entering memory-load contrast images (1-0; 2-0) into a 2 (Group: varenicline, placebo) x 2 (Time: Pre, Post) rmANOVA. To evaluate the impact of varenicline on negative emotional cue reactivity, BOLD response during the negative emotional vs. neutral trials will be the primary contrast of interest to test hypotheses that varenicline reduces negative affect and BOLD response in the corticolimbic negative affect pathway [e.g. rostral anterior cingulate, amygdala]. To evaluate the impact of varenicline on cannabis cue reactivity, BOLD response during the cannabis vs. non-cannabis trials will be the primary contrast of interest to test hypotheses that varencline reduces marijuana craving and BOLD response in mesocorticolimbic reward pathway [e.g. dorsal anterior cingulate (dACC), ventral striatum]. Similar to Froeliger et al. (2015), fMRI functional-connectivity data analyses will be performed with conn14 toolbox for SPM12, and hypothesis testing will be conducted using ROI-ROI explorer to characterize mean rZ values between ROIs of interest (e.g., R. IFG and thalamus). Where significant differences are observed, parameter estimates (from task-based models) and mean rZ values (from connectivity models) from each ROI will be extracted and relations with clinical endpoints examined. Additionally, parameter estimates and rZ values from each model will be evaluated as a potential

simple mediator in the efficacy pathway between varenicline and abstinence. Since the outcome variable (abstinence) in the mediation analysis is binary, we will rescale each coefficient according to the standard deviations of the predictor and outcome variables (MacKinnon et al., 1993). Small sample mediation has been shown to be unstable and thus we will resample with replacement 5000 times to create a bootstrapped confidence interval around the indirect effect (Bollen & Stine, 1990; Shrout & Bolger, 2002). When the indirect effect confidence interval does not contain zero, we will be able to state, with some certainty, that BOLD signal changes partially mediate the causal pathway between varenicline and cannabis abstinence.

Gender and smoking status will be explored as a potential moderators of study outcomes (i.e., clinical and fMRI) through model interactions. Medication adherence (MEMS cap, RedCap video, and self-report) will be assessed across treatment assignment as well as included as an additional variable in the primary efficacy and secondary craving/withdrawal models to examine any potential impact adherence may have on study outcomes. RedCap and MEMS cap data will be utilized as the primary (most stringent) markers of medication adherence, and we will secondarily conduct analyses of concordance between these data and self-report adherence.

Secondary Safety Analysis

Of particular interest will be adverse events leading to medication discontinuation and the occurrence of treatment-related serious adverse events (SAEs). We will specifically compare neuropsychiatric adverse events (assessed through psychiatric interview) using non-inferiority testing, as well as depression/anxiety (Hamilton Anxiety Scale [HAM-A] and Hamilton Depression Scale [HAM-D]) and suicidality (Columbia—Suicide Severity Rating Scale [C-SSRS]) ratings using one-sided 2-sample *t*-tests.

Participant Retention Analysis

Total number of treatment visits attended will be compared across treatment groups using a Poisson regression test, while the number of days retained will be assessed using Cox Proportional Hazards regression models.

Missing Data and Attrition. Missing data in longitudinal studies can be a problematic feature but can be mitigated through study design considerations. In order to minimize missing data and study attrition, design simplification and enhanced communication between study staff and participants will be emphasized. We will make every effort to prevent attrition (e.g., phone/text visit reminders, participation compensation, reinforcing adherence to the study protocol at each visit). In addition, in keeping with the Intent-to-Treat Principle, we will make every effort to continue assessments for the entire course of randomized treatment, even among those who fail to adhere to randomized assignment or stop participating in the study assigned intervention.

Continuation Milestone Criteria. The decision on whether or not to proceed with the subsequent UH3 fully-powered clinical trial will rest upon findings specific to the effect of varenicline, relative to placebo, on a number of key markers of efficacy. Specifically, we hypothesize that participants receiving varenicline, compared to those receiving placebo, will demonstrate attenuated levels of reported withdrawal-related negative affect at the end of the treatment portion of the study. This will provide evidence to support or refute our hypothesis regarding varenicline's mechanistic role in CUD. In randomized clinical trials assessing the efficacy of varenicline as compared to placebo for the reduction in smoking behavior, withdrawal and craving, Gonzales et al (2006) and Jorenby et al (2006) found that varenicline was superior to placebo in the reduction of withdrawal (as well as use and craving). Specific to withdrawal symptoms, those treated with varenicline as compared to placebo reported significantly less negative affect [Gonzales: Δ=-0.19; SEM=0.04; effect size (ES)=-0.30 and Jorenby: Δ=-0.13; SEM=0.04; ES=-0.21], less restlessness [Gonzales: Δ=-0.14; SEM=0.05; ES=-0.16 and Jorenby: Δ=-0.10; SEM=0.05; ES=-0.12], and reduced urges to smoke [Gonzales: Δ=-0.54; SEM=0.06; ES=-0.67 and Jorenby: Δ=-0.48; SEM=0.06; ES=-0.63] during study treatment. A) In keeping with these clinically relevant differences, we anticipate seeing effects equal to or greater than the more conservative of the two results (Gonzalez) in a CUD population. Thus, meeting or exceeding these criteria will be used as the threshold for clinically relevant evidence that would trigger justification to proceed with the UH3 trial. In addition to the primary outcomes, secondary clinical outcomes include both a reduction in cannabis use quantity and cannabis use abstinence at the end of the treatment portion of the study. We hypothesize a greater reduction in cannabis use quantity and greater cannabis abstinence will be recorded in the group randomized to varenicline as compared to placebo. B) A Cohen's d effect size of ≥0.4 in the secondary outcome of cannabis use reduction and/or an Odds Ratio of abstinence ≥1.5 in the varenicline group as compared to the placebo group will be used as an additional threshold

for clinically relevant evidence that would trigger justification to proceed with the UH3 trial. Meeting or exceeding the thresholds stated in A) and/or B) would provide the necessary clinical evidence justifying a fully powered efficacy clinical trial of varenicline as compared to placebo in the treatment of cannabis use disorder.

Power Calculation and Sample Size

The primary focus of the UG3 study is to assess whether varenicline, compared to placebo, will evidence equal or greater reductions in cannabis withdrawal-related negative affect during the final 4 weeks of treatment (after initial 2-week grace period, inclusive of medication titration and initial targeted quit date). Assuming a strong correlation between withdrawal and negative affect measures taken weekly within each subject (rho=0.8), a sample of n=68 participants (34 in each treatment group) will have adequate power (80%) to detect a clinically relevant effect size of d=0.60 between the two groups. With the stated sample size, similar between group differences (d=0.60) in weekly cannabis use quantity will be detectible between groups.

For the secondary abstinence analysis, the necessary sample size sufficient to estimate 50% of a fully-powered Phase 3 clinical trial for the abstinence endpoint will be determined. To show that treatment with varenicline will yield an abstinence rate at least 20% greater than placebo at the end of study treatment under the most conservative conditions, at a 15% placebo abstinence rate, a sample size of **n=72 participants** (**n=36 per group**) in each treatment assignment will provide 80% power with a type 1 error of 5% to detect this difference at the end of a fully-powered study. Thus, 50% of the study sample would require 36 participants per treatment arm in the UG3 portion of the study. Since we aim only to measure futility and do not plan on stopping for early efficacy, no alpha spending penalty has been incorporated into the study sample size.

Design Considerations

Comorbid tobacco use. As discussed above, individuals using both cannabis and tobacco have worse cannabis use treatment outcomes. Although data from a large RCT in alcohol using individuals found an effect of varenicline on drinking outcomes regardless of tobacco use status (Litten et al., 2013), it is possible that a differential effect of varenicline will be found among tobacco and non-tobacco using individuals with CUD. Therefore, in the initial UG3 trial, smoking status will be explored as a potential moderator of treatment response

Consideration of gender as a biological variable. In contrast to findings for stimulant drugs, there does not appear to be a strong effect of menstrual cycle phase on response to cannabis (for review, see Terner and de Wit, 2006). However, our recent work revealed a significant gender by treatment interaction in individuals with CUD, with women randomized to buspirone having fewer negative urine cannabinoid tests than women randomized to placebo (p=0.007), and men randomized to buspirone having significantly lower creatinine adjusted cannabinoid levels as compared to those randomized to placebo (p=0.023) (McRae-Clark et al., 2015). These findings support the need to consider gender as a critical variable in treatment investigations; as such, gender will be explored as a potential moderator of treatment response.

Inclusion of neuroimaging. Normalization of inhibitory control as well as reduction in cannabis cue reactivity may reflect key underlying mechanisms of varenicline's effect on CUD. Further, findings may help establish which individuals may respond optimally to varenicline treatment.

Timeline. The first three months will be used for staff training and preparing for study initiation. Eighteen months will be needed for participant recruitment and data collection. The final three months will be used for data analysis, determination of milestone accomplishment, and discussion with NIDA staff regarding progression to the UH3 phase. At a recruitment rate of approximately four participants per month (a rate consistently achieved in our prior studies), we anticipate no difficulty completing the study in this timeframe.

PROTECTION OF HUMAN SUBJECTS

RISKS TO THE SUBJECTS

a. Human Subjects Involvement and Characteristics

Admission into the study is open to men and women and to all racial and ethnic groups, age 18-65. Seventy-two individuals with cannabis use disorder will be recruited primarily through clinical referrals and internet and newspaper advertisements. Inclusion/exclusion criteria that apply to all participants are listed below:

General Inclusion Criteria

- Must meet DSM-5 criteria for cannabis use disorder and use cannabis at least 3 days per week in the last 30 days.
- Must be between the ages of 18 and 65 years.
- If female and of childbearing potential, must agree to use acceptable methods of birth control for the duration of the trial.
- Must consent to random assignment, and be willing to commit to medication ingestion.
- Must be able to read and provide informed consent.
- Must have body weight >110lbs (50kg) and have BMI between 18 and 35kg/m2
- Must function at an intellectual level and have knowledge of the English language to sufficiently allow for accurate completion of assessments.

Additional Inclusion Criteria for fMRI Eligibility

- Must be right-handed.

General Exclusion Criteria

- Women who are pregnant, nursing, or plan to become pregnant during the course of the study.
- Individuals with severe renal impairment (creatinine clearance less than 30 mL per minute).
- Lifetime history of DSM-5 Bipolar I or II Disorder, Schizophrenia or otherpsychotic disorder. Stably treated MDD, Dysthymia, GAD, Social Phobia, and Specific Phobia diagnoses are acceptable (i.e. same dose of medication has been prescribed for at least 2 months prior to screening and no changes in current medication expected during course of the trial).
- Suicidal ideation or behavior within the past 6 months. Subjects who are believed to be at suicidal or homicidal risk (answers 'yes' on questions 4 or 5 of C-SSRS) will be referred for assessment by a qualified mental health professional.
- Concomitant use of psychotropic medications, with the exception of stable doses (defined as no dosing adjustments in the past two months) of non-MAO-I antidepressants, non-benzodiazepine anxiolytics, and ADHD medications.
- Current use of medications prescribed for mania or psychosis.
- Current use of buproprion or nortryptiline.
- Moderate or severe non-cannabis substance use disorders within the past 60 days with the exception of tobacco use disorder.
- Individuals taking an investigational agent within the last 30 days before baseline visit.
- Individuals with clinically significant medical disorders or lab abnormalities.
- Any individual at screening with SGOT (AST) or SGPT (ALT) greater than 3 times the upper limit of normal and/or total bilirubin greater than two times the upper limit of normal.
- Individuals with clinically significant cardiovascular disease in the past 6 months (e.g., myocardial infarction, CABG, PTCA, severe or unstable angina, serious arrhythmia, or any clinically significant ECG conduction abnormality.
- Individuals with clinically significant cerebrovascular disease in the past 6 months such as TIA, CVA, or stroke.
- Hypersensitivity to varenicline.
- Individuals who have participated in the clinical trial of any investigative compound within the last 60 days.

Additional Exclusion Criteria for fMRI Eligibility

- Any psychiatric or medical issues, including claustrophobia, ferrous metal implants, pacemakers, or other electronic devices that would interfere with ability to participate in and successfully complete scanning procedures.
- Any person unable to lie still within the fMRI scanner for the required period of time to obtaine useful images (use of anxiolytics will not be permitted for anxiety/claustrophobia related to scanning procedures).

b. Sources of Materials

Research material obtained from individual participants includes questionnaires and interviews with study personnel, and breath, blood, and urine samples. To ensure confidentiality, all participant data will be letter/number coded, and only the investigators will have access to the master lists of codes. The research material will be obtained specifically for research purposes. Written research material obtained will be stored in the Addiction Sciences Division, in an office that is locked when not in use. Blood and urine samples will be stored in the Clinical Neurobiology Laboratory.

c. Potential Risks

The varenicline package insert details adverse events associated with the medication. Specifically, it reports that "the most common adverse reactions (>5% and twice the rate seen in placebo-treated patients) were nausea, abnormal (e.g., vivid, unusual, or strange) dreams, constipation, flatulence, and vomiting." Meta-analyses of the four main adverse events in varenicline versus placebo groups in adult trials yielded relative risks (RRs) of 3.21 (95% CI 2.71, 3.80) for nausea, 1.50 (95% CI 1.26, 1.79) for insomnia, 2.79 (95% CI 2.09, 3.72) for abnormal dreams, and 1.20 (95% CI 1.00, 1.45) for headache (Cahill et al., 2009). While post-marketing anecdotal reports of psychiatric adverse events led to an FDA "black box" warning for varenicline, a reanalysis of controlled trials revealed no evidence that varenicline is associated with neuropsychiatric adverse events (Thomas et al., 2015). There is a potential risk of loss of confidentiality. Exposure to cannabis cues and completion of the neurocognitive tasks may produce some craving for cannabis or other discomfort. However, this discomfort is usually brief and participants will be in the cannabis-free safety of a clinic environment. There is a chance that some of the pictures used in the negative affect task may cause some emotional distress. Since the scanner requires participants to be motionless in an enclosed environment, there is the possibility of a claustrophobic reaction or anxiety or discomfort secondary to being stationary for approximately 60 minutes per scan. Ferrous objects that are undetected could move during scans. This could lead to tissue damage and hemorrhage.

ADEQUACY OF PROTECTION AGAINSTS RISKS

a*. Recruitment and Informed Consent*

Participants will primarily be recruited through clinical referrals and the use of advertisements (internet, newspaper). Participants may also be recruited from the Ralph H. Johnson VAMC and the College of Charleston. Medical records will not be reviewed to identify potential study participants unless patients have requested to be contacted through a research permissions registry. A study PI, Co-I, or other qualified study staff will obtain informed consent. The informed consent form includes a detailed description of the study procedures, along with statements regarding participants' rights to withdraw from the procedure at any time without consequences. The informed consent form will be explained to participants in easy-to-understand language, and participants will be instructed to read the form carefully prior to signing it. Consent will be documented by the signature of the participant on the informed consent agreement, accompanied by the signature of the individual obtaining the consent.

b. Protections Against Risks

All study participants will be closely monitored for psychiatric and medical stability. All study procedures will be conducted under the supervision of experienced personnel. If crisis intervention is necessary during screening or study enrollment, senior staff will be available to evaluate the subject and provide an intervention or referral. In regards to suicidality, any participant endorsing suicidality on the C-SSRS (either during screening or at a subsequent study visit) will be assessed for safety by a psychiatrist. If hospitalization is indicated, the patient

will be hospitalized through MUSC or an appropriate referral will be made. All participants will be fully informed that they may withdraw from the study at any time without penalty.

To ensure confidentiality, all participant data will be coded by letters and/or numbers, and only the investigators will have access to the master lists of codes. All participant records will be kept in a locked cabinet in an office that will be locked at times when not in use. The research staff understands the importance of maintaining confidentiality, and this method of maintaining confidentiality has been used for several years by our research group and has been effective. All electronic databases are stored on HIPAA-compliant servers with restricted access. RedCap video clips will only be viewed by approved research staff and will be deleted when the study has ended and data analysis is complete. All co-investigators and study personnel have completed (or will complete upon hiring) training in Good Research Practices as mandated by the MUSC IRB.

Participants will be taught about potential side effects of varenicline and will be closely followed by psychiatrists, a PharmD, and other members of the research team. Pregnancy tests will be performed prior to medication initiation and monthly during the study. Participants will be excluded if they have a known hypersensitivity to varenicline. Adverse events will be assessed at each clinic visit, and all participants will be provided with an after-hours emergency contact number in the event that an adverse event occurs when the clinic is closed.

If abnormalities in the brain images are found, a participant will be immediately referred to an appropriate clinical care provider. A careful metal screening history will be taken from each participant to assess the possibility of metal devices. Individuals will be screened with a metal detector for the possibility of implanted metal objects since magnetic movement of a metal device or metal injury could result in injury or risk to life. Prior exposure to pictures of the scanner, getting into the scanner, and seeing others in the scanner often reduces psychological discomfort or identifies people for whom scanning is not appropriate. Subjects are able to ask study staff to stop the negative affect scanning task at any time if they feel too upset by the pictures.

POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECT AND OTHERS
Possible risks to study participants include adverse reactions to varenicline and risks associated with fMRI scanning. Benefits include detailed assessment of cannabis use and receipt of an evidence-based psychosocial treatment for cannabis use disorder. The minimal risks are reasonable in relation to the potential benefits to be gained from the investigation.

IMPORTANCE OF THE KNOWLEDGE TO BE GAINED
This study may help accelerate approval of varenicline for treatment of cannabis use disorder. Presently, there are no FDA-approved medications for cannabis use disorder. The moderate risks of the investigation are considered reasonable in relation to the expected knowledge to be gained.

CLINICALTRIALS.GOV REQUIREMENTS
In accordance with Public Law 110-85, this project will be registered at the ClinicalTrials.gov Protocol Registration System Information Website prior to study initiation.