Protocol Title: A Novel Compound for Alcoholism Treatment: a Translational Strategy – Part II

Protocol Number: 16-DA-0080

Date of This Submission/Version 8/21/2021version 5.3 Amend M

Total requested accrual: 55 subjects

Project Uses Ionizing Radiation: ☒No ☐Yes (*attach RSC/RDRC documentation)*

- ☐ Medically-indicated only
- ☐ Research-related only
- ☐ Both

IND/IDE ☐No ☒Yes *(attach FDA documentation)*

Drug/Device/ # 119,365 (cross-reference Pfizer IND # 110,476)

Sponsor: National Institute on Alcohol Abuse and Alcoholism (NIAAA): IND Withdrawn by the NIAAA OCD August 2021

Durable Power of Attorney ☒ No ☐ Yes

Multi-institutional Project ☒ No ☐ Yes

Data and Safety Monitoring Board ☐No ☒Yes

Technology Transfer Agreement × No Yes

Agreement type and number

Samples are being stored ☐ No ☒ Yes

Flesch-Kincaid reading level of consent form: 9.2

A. **Précis:**

Objective: Ghrelin is a 28-amino acid peptide that stimulates appetite and food intake. It is an endogenous ligand for the growth hormone secretagogue receptor (GHSR1a). Preclinical studies suggest that ghrelin modulates alcohol reward processing. Previous work from our research team, indicated that intravenous (IV) ghrelin administration, compared to placebo, results in an acute increase in alcohol craving during a cue-reactivity experiment in alcoholic individuals. Therefore, an oral bioavailable, ghrelin receptor antagonist that is able to pass through the blood brain barrier holds particular promise as a treatment for alcohol use disorder (AUD). This protocol is part of a grant project funded by National Center for Advancing Translational Sciences (NCATS) aimed to generate preliminary evidence in AUD on the safety and efficacy of a ghrelin receptor (GHSR1a) antagonist, PF-05190457, an existing molecule available under the NIH-Industry Pilot Program at NCATS. Completed preclinical and clinical (Protocol #14-AA-0042) work has demonstrated the safety of PF-05190457/alcohol interaction. The goal of this protocol is to conduct a proof-of-concept human laboratory study to assess an early-signal of efficacy of PF-05190457 in AUD.

Study population: The study population will be AUD individuals (n = 55).

Study Design: A within-subject, counterbalanced, double-blind, placebo-controlled study.

Outcome measures: The primary aim will be to determine whether PF-05190457, compared to placebo, reduces alcohol cue-elicited craving. The main secondary aim will be to determine whether PF-05190457, compared to placebo, reduces brain blood oxygen level dependent (BOLD) response during exposure to alcohol cues, during a task-based fMRI scan. We will also investigate the effects of PF-05190457 on food craving as well as on food choices using a “virtual buffet” experimental procedure. All these outcomes will be assessed in the inpatient Unit at the NIH CC. After the inpatient portion of the protocol, patients will be followed-up as outpatients. During the outpatient phase, patients will be offered motivational interviewing and video feedback to explore the effects of this intervention, compared to supportive counseling, on maintaining motivation for alcohol abstinence and inform future studies where medications like PF-05190457 and behavioral treatments may be combined. The outpatient phase is optional for treatment seeking and nontreatment seeking participants.

B. **Table of Contents**

C. **Background**

Statement of the problem: searching for new neuropharmacological targets in alcohol use disorder

Alcohol use disorder (AUD) afflicts ~10% of the US population, and causes serious morbidity and mortality (Miller et al., 2001; NIAAA, 2000). Treatment of AUD consists of psychological, social and pharmacologic interventions (Garbutt et al., 1999). When combined with psychosocial treatments, medications can improve outcomes for some individuals; however, these treatments are unsuccessful for many others (Heilig & Egli, 2006; Edwards et al., 2011). The Food and Drug Administration (FDA) has only approved disulfiram, naltrexone and acamprosate for AUD, and all these medications have limited efficacy (Heilig & Egli, 2006; Edwards et al., 2011). Developing new drugs for AUD is therefore a high scientific and public health priority. There is a crucial need to identify novel drug targets that may lead to the discovery of new effective medications for AUD. One source of new drug targets comes from research on the neurobiology of obesity, as alcohol- and food-seeking behaviors have common features and neural circuits (reviewed in Tomasi & Volkow, 2012). While alcoholism, obesity and binge eating are complex conditions with likely diverse genetic and environmental contributions to their etiologies (Tomasi & Volkow, 2012), it is notable that medications used to treat AUD often result in weight loss. For example, naltrexone, topiramate and ondansetron have all been utilized to treat obesity and/or eating disorders (Leggio et al., 2011). Therefore, research focused on identifying feeding-related pathways may be of utility in understanding the neurobiology of alcoholism, and may yield possible neuropharmacological targets for AUD. As outlined below, there is an increasing body of evidence for the ghrelin system as a novel neuropharmacological target for AUD.

What is ghrelin and what does it do?

Ghrelin was first isolated from the stomach (Kojima et al., 1999). It is a 28-amino acid peptide acting as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R1a), a G-protein coupled receptor that induces growth hormone (GH) release from the pituitary (Kojima et al., 1999). The n-octanoyl bearing ghrelin is known as active ghrelin (acylated), although the des-acylated ghrelin is not totally inactive (Stengel & Taché, 2009). Ghrelin activates hypothalamic orexigenic neurons and inhibits anorectic neurons to induce hunger (Toshinai et al., 2003; Solomon et al., 2005); accordingly, intracranial ghrelin administration stimulates feeding in mammals (Cowley et al., 2003; Tschop et al., 2000) and non-mammals (Furuse et al., 2001; Unniappan et al., 2002). In humans, intravenous (IV) ghrelin administration increases appetite and food intake (Druce et al., 2005; Neary et al., 2004; Akamizu et al., 2008). Ghrelin stimulates appetite and food intake by acting on the arcuate nucleus (ARC). Opioidergic neurons play a role in alcohol reinforcement and are also located in the ARC (Loose et al., 1991). The highest expression of GHS-R1a's is in the central nervous system (CNS). GHS-R1a is also expressed in several peripheral tissues such as the stomach, intestine, pancrease, thyroid, gonads, adrenal, kidney, heart and vasculature. It is unclear how circulating ghrelin reaches CNS targets. It may do so by crossing the blood-brain barrier (BBB) or by direct diffusion (or passage) as the BBB is incomplete at the ARC (Chollet et al., 2009). Local CNS regulation may occur as small quantities of ghrelin are produced in the hypothalamus and thus, may also regulate neurons expressing GHS-R1a's (Carpino et al., 2002).

Rationale for studying the ghrelin system in alcohol use disorder

In addition to the ARC, GHS-R1a's are also highly co-expressed with dopamine (DA) receptors in other brain regions (e.g., dentate gyrus of the hippocampus, midbrain, substantia nigra, raphe nuclei and ventral tegmental area [VTA]) (Guan et al., 1997; Katayama et al., 2000; Zigman et al., 2006; Jiang et al., 2006), suggesting that ghrelin modulates reward processing (Jerlhag et al., 2006a; 2007). Feeding behavior, DA release and locomotor stimulation are triggered by ghrelin infusion after intracerebral ventricular (ICV) infusion and after peripheral ghrelin administration

(Naleid et al., 2005; Jerlhag et al., 2006b; Kawahara et al., 2009; Quarta et al., 2009). Alcohol- and food-seeking behaviors share common neurobiological mechanisms, and both alcohol and food exert their reinforcing effects, in part, by increasing DA in limbic regions (reviewed in: Leggio et al., 2011; Tomasi & Volkow, 2012). Cortico-mesolimbic DA pathways may mediate alcohol's rewarding effects (including craving) associated with its abuse liability (reviewed in: Koob, 1992; Tupala & Tiihonen, 2004). Thus, involvement of ghrelin in the DA reward system and the role of the DA reward system in AUD suggest a significant role of ghrelin in the pathophysiology of AUD, as detailed in the animal and human studies summarized below.

Animal studies:

Ghrelin modulates cholinergic and dopaminergic reward pathways

Ghrelin administration into the VTA increases extracellular concentrations of accumbal DA in mice (Jerlhag et al., 2007). Peripheral administration of ghrelin or direct administration into the intra-laterodorsal tegmental area (LDTg) concomitantly increases release of ventral tegmental acetylcholine and accumbal DA in rats (Jerlhag et al., 2012). The non-selective nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine attenuates the stimulatory and DA-enhancing effects of ghrelin infused into the third ventricle (Jerlhag et al., 2006b), indicating that nAChRs mediates these neurochemical properties of ghrelin; nAChRs also mediate the locomotor stimulatory and DA-enhancing properties of ghrelin administered into the VTA (Jerlhag et al., 2008). Only alpha3-beta2, beta3, and/or alpha6 nAChR subtypes are implicated in the central alcohol rewarding actions of ghrelin (Jerlhag et al., 2008). The same nAchRs (alpha3-beta2, beta3, and/or alpha6) in the VTA mediate the rewarding properties of alcohol, modulating voluntary-ethanol-consumption induced increases in both VTA acetylcholine and accumbal DA levels (Larsson et al., 2004; 2005). Peripherally injected ghrelin also activates reward-related measures, such as locomotor activity, accumbal-DA release and conditioned place preference (CPP), an established measure of alcohol reward where mice are conditioned to associate an environment with previous alcohol exposure in the absence of alcohol itself (Jerlhag, 2008). In summary, these studies demonstrate that both ghrelin and ethanol share a common substrate, the cholinergic-dopaminergic reward system, for the rewarding properties of ethanol.

Ghrelin is required for alcohol reward

Another set of experiments demonstrated that ICV ghrelin administration to mice significantly increased alcohol intake compared to vehicle treatment in a 2-bottle (alcohol/water) free choice limited access paradigm, and this increase was even more robust when ghrelin was administered bilaterally into either the VTA or the LDTg (Jerlhag et al., 2009). The effects of ghrelin on the VTA and LDTg were specific for alcohol intake (i.e., food intake was increased by ghrelin ICV in comparison to vehicle but was not affected by bilateral ghrelin administration into either the VTA or the LDTg). Notably, ghrelin administration into the lateral hypothalamus or paraventricular nucleus had no effects on ethanol intake (Schneider et al., 2007), confirming that ghrelin works in specific brain reward nodes (i.e., VTA). Furthermore, the effects of ICV ghrelin on alcohol intake were absent in GHSR knockout mice (Jerlhag et al., 2009). Additional experiments reported that the rewarding properties of alcohol (i.e., alcohol-induced accumbal DA release and locomotor stimulation) are also attenuated in ghrelin knockout mice compared to wild type (Jerlhag et al., 2011). In summary, these studies demonstrate that central ghrelin signaling is required for alcohol reward.

*Specificity of Ghrelin receptor (*GHS-R1a*) antagonism for reduction of alcohol reward and consumption*.

Another set of experiments done in Jerlhag and Engel's lab demonstrated that measures of alcohol reward, such as enhanced extracellular accumbal DA overflow, locomotor stimulation and CPP were consistently abolished or

attenuated by two GHS-R1a antagonists (BIM28163 and JMV2959) in wild-type mice and were abolished in GHS-R1a knockout mice (Jerlhag et al., 2009). Alcohol intake was suppressed in mice by both BIM28163 and JMV2959 [delivered either ICV or intraperitoneal (IP)]. These effects were specific for alcohol intake, as: a) JMV2959 administration resulted in only a slight decrease in food intake, while BIM28163 induced an increased (rather than a decrease) in food intake, probably via an unknown mechanism that is likely to be independent of GHS-R1a; and b) GHS-R1a antagonists abolished CPP (see: Sanchis-Segura & Spanagel, 2006), thus demonstrating that the role of ghrelin signaling in alcohol reward is independent of the caloric value of alcohol (Jerlhag et al., 2009). Additional experiments with Long-Evans rats and alcohol preferring [Alko alcohol (AA)] rats reported that JMV2959 reduced high alcohol intake as well as the motivation to consume alcohol; i.e., operant self-administration (Landgren et al., 2012). In summary, pharmacological studies demonstrate that GHS-R1a antagonism results in reduced alcohol reward and consumption.

GHS-R1a antagonism inhibits both evoked and spontaneous GABAergic activity in the central nucleus of the amygdala (CeA)

Cruz and colleagues (2013) used quantitative reverse transcription polymerase chain reaction to demonstrate the presence of GHS-R mRNA in the CeA (an area with a key role in regulating ethanol consumption whose GABAergic transmission is enhanced by acute and chronic ethanol administration) and used electrophysiological methods to demonstrate tonic ghrelin signaling in the CeA (Cruz et al., 2013). In naïve animals, superfusion of ghrelin increased the amplitude of evoked inhibitory postsynaptic potentials (IPSPs) and the frequency of miniature inhibitory postsynaptic currents (mIPSCs); co-application of ethanol further increased the ghrelin-induced enhancement of IPSP amplitude. In chronic ethanol-treated animals, superfusion of the GHS-R1a antagonists D-Lys3-GHRP-6 and JMV 3002 decreased evoked IPSP and mIPSC frequency, revealing tonic ghrelin activity in the CeA. Blockade of GHS-R1a receptors with D-Lys3-GHRP-6 and JMV 3002 had a significant inhibitory effect on both evoked and spontaneous GABAergic activity, suggesting constitutive activation of GHS-R1a's or tonic activity of endogenous ghrelin signaling in rat CeA. Pretreatment of CeA neurons with the GHS-R1a antagonists completely blocked the ghrelin-induced facilitation of IPSP amplitudes, indicating that ghrelin exerts its effect through GHS-R1a's. These results suggest that the ghrelin system may constitute part of a brain pathway modulating reinforcement properties of alcohol consumption, and provide additional evidence for the potential role of ghrelin receptor antagonism as a novel pharmacological approach to treat AUD.

Human studies:

Blood ghrelin levels, alcohol intake and craving

In healthy volunteers, blood ghrelin levels are significantly reduced after acute alcohol consumption (Calissendorff et al., 2005, 2006, 2012; Zimmermann et al., 2007). Additional studies reported that non-abstinent alcoholics had lower blood ghrelin concentration compared to controls (Addolorato et al., 2006; Badaoui et al., 2008; de Timary et al., 2012), and abstinent alcoholics had increased levels (Kim et al., 2005; Kim et al., 2012; Kraus et al., 2005). Together, these studies suggest that ghrelin levels are suppressed by acute alcohol intake and increased during abstinence. The PI, Dr. Leggio, conducted a study that provided the first preliminary evidence of a significant positive correlation between blood ghrelin levels and the Obsessive-Compulsive Drinking Scale (OCDS) craving scores in active drinking alcoholic individuals (Addolorato et al., 2006), a finding also confirmed by another recent study (Koopmann et al., 2012). Subsequent studies partially confirmed the relationship between blood ghrelin levels and alcohol craving in alcoholic individuals, albeit only in those with positive family history of alcoholism (Hillemacher et al., 2007) or in females (Wurst et al., 2007). Dr. Leggio led the first longitudinal study of serum ghrelin levels in alcoholic patients over the course of 12 weeks treatment. Ghrelin concentrations were assessed at

baseline (T0; after 72 hours abstinence), then repeatedly at 2 weeks (T1), 6 weeks (T2), and 12 weeks (T3); levels in abstinent patients were compared to those who did not remain abstinent during the 12 weeks treatment. At baseline, blood ghrelin levels were significantly higher in the non-abstinent group (p=0.035) and there was a significant *group x time* interaction in ghrelin levels (F=4.193, df=3, p=0.012), [Figure 1 (Leggio et al., 2012)]. These findings suggest that ghrelin level in early abstinence predicts later relapse. We also found a significant positive correlation between baseline ghrelin levels and craving during the 12-week period (Leggio et al., 2012). In summary, consistent with the preclinical data, human studies show that higher ghrelin levels may be associated with higher craving and alcohol consumption, suggesting that ghrelin is a potential new pharmacological target for AUD. Although only peripheral levels of ghrelin were tested, these results support the hypothesis that ghrelin may influence alcohol craving and consumption via the DA-related rewarding processing. This is consistent with a PET study (although in a different population of obese subjects) showing that peripheral ghrelin levels were inversely associated with the availability of DA type 2 receptor (D2R) in the caudate, putamen, ventral striatum, amygdala, and temporal lobes, i.e., higher ghrelin levels were associated with lower D2R availability, presumably from increased DA release and receptor occupancy (Dunn et al., 2012). Notably, although only peripheral ghrelin levels were tested, circulating ghrelin may reach CNS targets directly by crossing the BBB (Chollet et al., 2009).

Figure 1

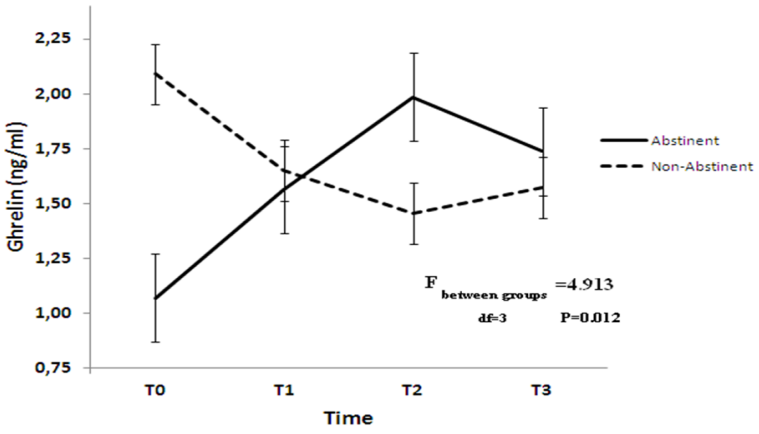


Figure 1. Differences in blood ghrelin levels between abstinent and non-abstinent alcoholic patients.

Human ghrelin administered intravenously acutely increases alcohol craving: first-in-man preliminary findings

Dr. Leggio conducted the first study administering IV ghrelin to alcoholic individuals (NCT01190085: PI Leggio). In this double-blind, placebo-controlled, between-subject, randomized study, 45 non-treatment seeking heavy drinking alcoholic individuals received a bolus of IV ghrelin 1 μg/kg, IV ghrelin 3 μg/kg or saline solution (placebo) and then immediately participated in a cue-reactivity (CR) experiment in a controlled laboratory setting. During the CR procedure, participants were exposed to alcohol cues (e.g., the sight and smell of one's preferred alcoholic beverage), compared to control cues (while water is usually used in CR studies, juice cues were used in this study to specifically control for non-alcoholic appetitive behaviors). Both subjective (i.e., urge to drink and attention to the cues) and physiological (i.e., heart rate, blood pressure, salivation) responses to cues were collected. The Alcohol Urge Questionnaire (AUQ) was used to assess urge to drink alcohol.

The main results of this study (Leggio et al., 2014) showed a main ghrelin effect in increasing the urge to drink alcohol [$F(2,40) = 3.36$, $p = .045$] using a repeated measures ANCOVA (Figure 2). Post-hoc comparisons revealed that alcohol urge

was significantly greater for ghrelin in 3 mcg/kg than placebo (p = .046) (Figure 2), with a large effect size (d = 0.94). No statistically significant differences were found neither in the ghrelin 1 mcg/kg vs. placebo condition nor between ghrelin 1 mcg/kg and ghrelin 3 mcg/kg. In contrast with urge to drink alcohol, IV ghrelin was not significantly more effective than placebo in increasing urge to drink juice.

Figure 2

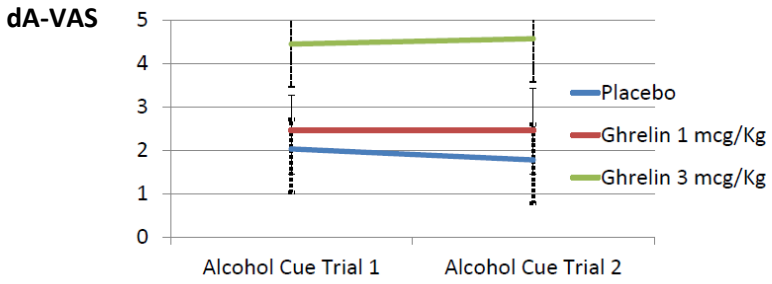


Figure 2. Intravenous ghrelin 3 mcg/kg administration, compared to placebo, resulted in increased urge to drink alcohol, measured as change in an Alcohol-Visual Analogue Scale (dA-VAS) during a human laboratory study with alcoholic individuals. There was a main ghrelin effect in increasing the urge to drink alcohol [F(2,40) = 3.36, p = .045] using a repeated measures ANCOVA. Post-hoc comparisons revealed that alcohol urge was significantly greater for ghrelin 3 mcg/kg than placebo (p = .046), with a large effect size (d = 0.94).

Post-infusion serum total ghrelin level was correlated with the increase in alcohol urge during both the first (p = .008, Figure 3A) and the second (p = .005, Figure 3B) alcohol trials. Similarly, the maximum serum ghrelin peak level across the six measurements correlated with the increase in alcohol urge during both the first (p = .02, Figure 3C) and second (p = .04, Figure 3D) alcohol trials. By contrast, neither urge to drink juice nor food craving questionnaire scores were significantly

correlated with serum ghrelin levels.**Figure 3**

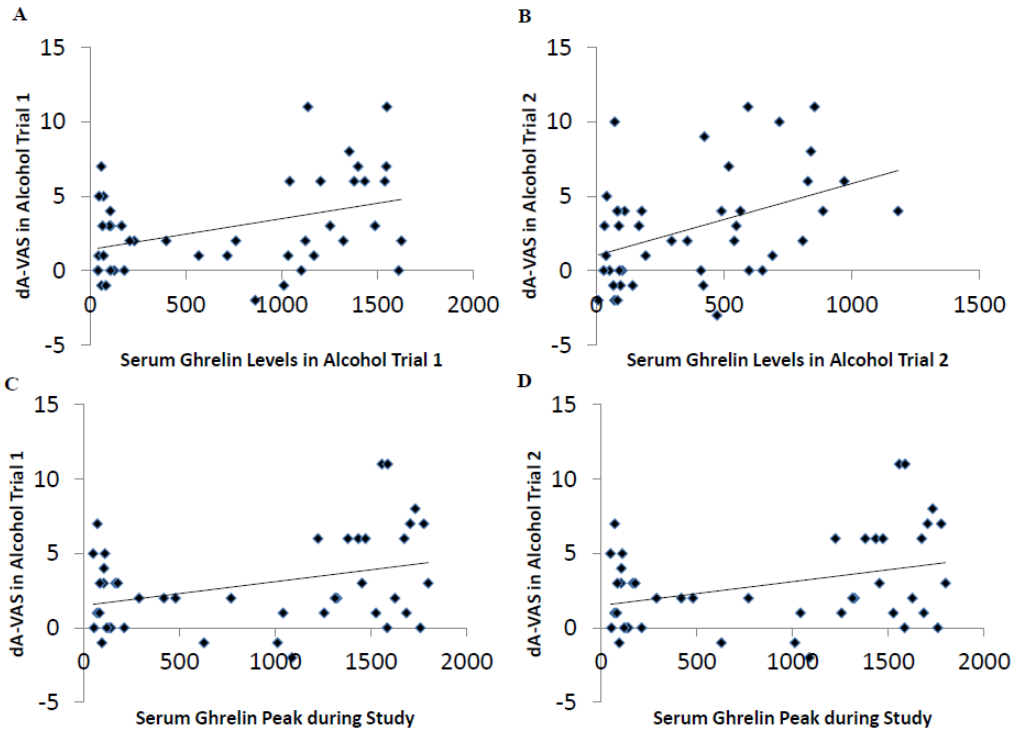


Figure 3. Serum total ghrelin level was correlated with the increase in alcohol urge measured by Alcohol-Visual Analogue Scale (dA-VAS) during both the first (p = .008; Figure 3A) and the second (p = .005; Figure 3B) alcohol cue trials. The maximum serum total ghrelin peak level across the six measurements was correlated with the increase in alcohol urge both in the first (p = .02; Figure 3C) and the second (p = .04; Figure 3D) alcohol cue trials.

Albeit preliminary, these data have clinical implications given that craving is a predictor of alcohol use. Craving has been proposed as a clinically relevant endophenotype, as higher craving is associated with an increased rate of relapse (Marlatt, 1978; Rohsenow et al., 2001; Bottlender & Soyka, 2004). Furthermore, the CR procedure is well-validated (e.g., Monti et al., 1993, 2000; Rohsenow et al., 2000; Leggio et al., 2013). Exposure to alcohol cues can simulate a high risk situation for relapse and urge to drink, as assessed in the CR, and predict drinking after treatment (Monti et al., 2000). Notably, medications (e.g., naltrexone) that reduce alcohol consumption also reduce alcohol craving during the CR procedure (Monti et al., 2000; Swift, 1999).

GSHR1a antagonism/inverse agonism: a novel pharmacological strategy to treat AUD

The studies summarized above provide compelling evidence that manipulations of the ghrelin system affect alcohol craving and consumption. Both animal and clinical studies show that ghrelin administration results in increased craving and drinking and there is direct preclinical evidence that GHS-R1a antagonism significantly reduces alcohol intake. Thus, orally bioavailable, brain penetrant GHS-R1a antagonists may have a therapeutic promise for AUD (Jerlhag et al., 2009; Leggio, 2010; Leggio et al., 2012), but this hypothesis has not yet been tested in humans. The development of GHS-R1a antagonists for other medical conditions (e.g., obesity) is under way, but most of them are neither ready for 'first-in-man' use nor available to academic investigators. This proposal offers a unique opportunity to test, for the first time, this hypothesis using PF-05190457, an existing molecule available under this NIH-Industry Pilot Program at NCATS, and with proven safety in Phase 1 studies. It has been shown that clinically effective antagonists (especially competitive antagonists) are frequently

inverse agonists rather than simply neutral antagonists. Chollet et al. (2009) reported that GHS-R1a possesses a naturally high ligand-independent signaling representing 50% of its maximal activity and this property implies that a neutral antagonist could have limited benefit compared with an antagonist possessing inverse agonist activity (Pantel et al., 2006; Holst & Schwartz, 2006). Consistent with this notion, GHS-R1a inverse agonists could be effective for obesity, in that they would be expected to eliminate the high constitutive, ghrelin independent signaling activity of GHS-R1a between meals (Holst & Schwartz, 2004); thus, GHS-R1a inverse agonists may decrease the craving for 'second-order meals' (Holst & Schwartz, 2004). Similarly, GHS-R1a inverse agonism may represent an effective way to manipulate ghrelin signaling to reduce alcohol craving and relapse in heavy drinkers (Leggio, 2010). PF-05190457 is a GHS-R1a inverse agonist/competitive antagonist and represents, therefore, a promising new medication for AUD (Haas, 2012). Access to PF-05190457 via this NIH-Industry Pilot Program (NCATS, 2012) represents a unique opportunity to test, for the first time in humans, the hypothesis that competitive antagonism with inverse agonism activity on GHS-R1a may result in reduced alcohol craving and intake in individuals with AUD.

PF-05190457: PHARMACOLOGICAL PROPERTIES AND SAFETY PROFILE IN PHASE 1 STUDIES

PF-05190457 is a potent and selective first in class GHS-R1a inverse agonist/competitive antagonist originally proposed by the manufacturer (Pfizer) for the treatment of Type 2 diabetes mellitus based on its ability to increase insulin secretion from human islets. Preclinical and clinical studies on PF-05190457 have been conducted by Pfizer, which has agreed to partnership with NIAAA and URI for the execution of this proposed research. This paragraph briefly summarizes the available data for PF-05190457, based on the Confidentiality Disclosure Agreements (CDAs) between Pfizer and both NIAAA and URI. This project will be performed as part of an NIH/NCATS UH2/UH3 grant awarded to Dr. Leggio (NIH) and Dr. Akhlaghi (URI) (Co-PIs).

Absorption, distribution and metabolism

Oral absorption of PF-05190457 following single dose administration was rapid in rats and dogs, reaching T_{max} in less than 2 hours in both species. Oral bioavailability was moderate in rats (29.4%) and high in dogs (79.5%). PF-05190457 plasma protein binding, at concentrations of 1 μM and 10 μM, was moderate and independent of the concentration. Average values of unbound fraction were 0.34 in rat, 0.4 in dog, 0.16 in monkey, and 0.15 in human plasma. Clearance (CL), volume of distribution at steady state (V_{ss}), and elimination half-life ($t_{1/2}$) for PF-05190457 in humans was approximately 3.30 mL/min/kg, 1.94 L/kg, and 6.79 hours, respectively. Preliminary data suggests that PF-05190457 metabolism is catalyzed by CYP3A isoenzymes (3A4 and 3A5). PF-05190457 is not anticipated to demonstrate competitive or time-dependent pharmacokinetics drug interactions with compounds for which CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A mediated metabolism constitutes the primary mechanism of clearance. In cryopreserved human hepatocyte culture, PF-05190457 did not induce CYP3A or CYP1A2 isoenzymes. Food does not significantly affect PF-05190457 pharmacokinetics; therefore the drug can be taken either with or without food.

Pharmacodynamic properties

PF-05190457 has a human equilibrium dissociation constant for ligand and receptor interaction (Kd) of 3.04 nM (1.56 ng/mL) and requires 4.07 hours to achieve equilibrium in vitro. Based on the nonclinical studies, the cardiovascular and central nervous systems have been identified as the major potential targets, including a central effect of PF-05190457 in decreasing locomotor activity. Nonclinical toxicology data support the use of PF-05190457 in clinical studies up to 28 days in duration.

Altered cytochrome P450 (CYP) 3A4 in alcoholic subjects

CYP3 A4 is the most important drug-metabolizing enzyme in the liver and is responsible for biotransformation of 55% of all marketed medications (Anzenbacher & Anzenbacherova, 2001). Alcohol consumption is considered one of the underlying factors responsible for the variation in the induction of CYP3A4 and its baseline activity (He et al., 2006; Niemela et al.,

2000; Parkinson et al., 2004; Rahmioglu et al., 2011; Salmela et al., 1998; Weathermon & Crabb, 1999). Although alcohol dehydrogenase and CYP2E1 are the major pathways for metabolizing ethanol, CYP1A2 and 3A4 also contribute to alcohol metabolism (Salmela et al., 1998). A small-scale study of liver biopsies from patients with alcoholic and non-alcoholic liver disease showed that alcohol-induced liver damage results in the induction of CYP2A6, CYP2E1 and CYP3A4 isoforms (Niemela et al., 2000). Recently, a classical twin study investigated the influence of genetic and environmental factors on CYP3A4 induction. Induced CYP3A4 activity was estimated at 66% because of genetic factor while BMI, alcohol use and cigarette smoking accounted for 20% of the variability in CYP3A4 induction (Rahmioglu et al., 2011). Furthermore, in human liver, data obtained in donors with a history of alcohol consumption (14 or more drinks per week) showed a 2.2 fold higher rate of metabolism of midazolam to its 1'-hydroxy metabolite that suggests induction of CYP3A4 by alcohol (He et al., 2006). Midazolam, is a specific CYP3A4 substrate for both in vitro and in vivo studies. Contrary to this observation, Swart et al. (2004) showed 35% lower clearance of midazolam in patients with a history of alcohol abuse defined as chronic use of more than 6 units of alcohol per day. Midazolam volume of distribution was not affected by alcohol abuse (Swart et al., 2004). In addition, the formation of autoantibodies against CYP2E1 and CYP3A4 was detected in alcoholic subjects and formation of IgG against these enzymes was evident in rat during chronic ethanol feeding (Lytton et al., 1999). Alcohol consumption is also associated with reduced responsiveness to anti-retroviral agents (Samet et al., 2003). A spectral binding study showed that ethanol physically interacts with CYP3A4 protein with a strong binding affinity. As a result, the inhibitory effect of a CYP3A4 inhibitor, nelfinavir, was 3-fold lower in the presence of 20 mM ethanol (Kumar et al., 2010; Kumar & Kumar, 2011).

P-glycoprotein as a barrier for PF-05190457 brain penetration

The blood capillary endothelial cells in the brain form tight junctions that are impermeable to many molecules. Generally, hydrophilic compounds like glucose, ethanol and nicotine pass across blood-brain barrier (BBB) more easily via passive diffusion (Schinkel, 1999), compared to relatively large (> 400 Da) hydrophobic drugs. PF-05190457 (Mw: 512.68 Da; Log P: 2.93) is a brain penetrant compound, as evidenced by somnolence at higher doses, but it is technically defined by the manufacturer as a 'brain impaired' compound given that the brain/plasma ratio is not 1:1. P-glycoprotein (P-gp) encoded by multidrug resistance protein 1 (MDR1) gene is an ATP-dependent efflux transport protein that is predominantly found in the apical membranes of cell in intestine, liver, kidney and BBB. In vitro experiments and in vivo studies in P-gp knockout animals suggest that efflux by P-gp is responsible for poor brain penetration of relatively large hydrophobic drugs (Schinkel, 1999). In vitro studies carried out by Pfizer have shown that PF-05190457 is a substrate for P-gp. In addition, the brain to plasma free concentration ratio of PF-05190457 in P-gp knockout mouse was 2.534 as compared to 0.016 in the wild type mice.

INNOVATION

Since 2006, the preclinical and clinical literature on the role of ghrelin and its receptor in AUD has been exponentially increasing. As such, targeting the ghrelin receptor system to treat AUD is a highly innovative approach. The most translational study conducted in humans with ghrelin is Dr. Leggio's study where alcoholic individuals received IV ghrelin. However, no studies have ever been conducted testing a ghrelin receptor antagonist in alcoholic individuals. Therefore, this study partially funded by a UH2/UH3 mechanism from NCATS represents a highly innovative project because it will be the first clinical trial testing the possible role of a ghrelin receptor antagonist/inverse agonist in AUD. While a few preclinical experiments with other ghrelin receptor antagonists have been conducted, our pre-clinical and phase 1b studies with PF-05190457 proved this compound to be safe in AUD patients. The objective of phase 2a study will be to determine whether PF-05190457, compared to placebo, reduces alcohol cue-elicited craving, reduces brain blood oxygen level dependent (BOLD) response during exposure to alcohol cues and during a task-based fMRI scan is highly translational and will provide pivotal information to support further development of a novel compound for treatment of AUD.

Summary of previous Phase 1a clinical studies of PF-05190457 conducted by the manufacturer

Pfizer has conducted three Phase 1 clinical trials showing that PF-05190457 is safe and generally well tolerated up to 100 mg twice a day (b.i.d.) for 14 days. The first study, B3301001 (www.clinicaltrials.gov : NCT01247896), was a Phase 1 placebo-controlled trial to assess the safety, tolerability, and PK of single escalating oral doses of PF-05190457 in 35 healthy adult subjects. Following the administration of a single dose to fasting subjects, PF-05190457 absorption was rapid, with median T_{max} values observed between 0.500 and 2.50 hours across the studied doses, and these values decreased with increasing the dose. Median plasma PF-05190457 concentrations increased with increasing doses, consistent with the individual concentration-time profiles. In general, increase in exposure (AUC_{last} and C_{max}) to PF-05190457 was dose proportional within the range of 50 mg to 300 mg. This study indicated a safe profile of PF-05190457. There were no serious adverse events (SAEs), no subject discontinued due to an AE, and no dose reductions or temporary discontinuations of study medication occurred due to an AE. The maximum tolerated single dose was determined to be 300 mg based on treatment-related observations of heart rate increases and somnolence. The most common AEs were somnolence, headache, and fatigue. All somnolence events were mild, and no AEs were graded as severe. A PF-05190457 plasma concentration-related heart rate increase was also observed. There was also a tendency for the drug to lower glucose levels.

The second study, B3301007 (www.clinicaltrials.gov : NCT01522807), was another Phase 1 study aimed at testing the bioavailability of three different formulations of PF-05190457 100mg in 16 healthy adult volunteers: EP-suspension formulation, long duration, and short duration EP-osmotic capsule formulations. This second trial confirmed the safe profile of PF-05190457, e.g., no SAEs or severe AEs. Heart rate increase and somnolence were again the most common AEs.

The third study, B3301002 (www.clinicaltrials.gov : NCT01372163), was a Phase 1 study testing multiple doses of PF-05190457 (up to 100mg b.i.d.) in healthy controls and diabetic patients during a 14-day period. This study confirmed the safety of the drug, and as stated on www.clinicaltrials.gov the study was terminated "*for strategic reasons, there were no safety concerns leading to discontinuation of this study*." In the third study (B3301002), tachyphylaxis was observed, affecting the ghrelin-stimulated GH secretion, increases in heart rate and glucose-lowering tendency by Day 14. Interestingly, in B3301002, after multiple dosing of PF-05190457 100mg b.i.d. (the dose we propose in our HU3 clinical trial), somnolence was still evident but the heart rate effects showed tachyphylaxis presumably because heart rate is vagally–mediated and PF-05190457 at 100 mg b.i.d. blocks the receptor 24/7, whereas the somnolence driven centrally did not have a 24/7 block of the receptor. This observation further confirms that PF-05190457 acts centrally in the CNS and that, unlike its peripheral actions, the central actions of PF-05190457 are less likely to show tachyphylaxis. Nonetheless, the tachyphylaxis observation made in the B3301002 study was carefully considered in our approach and human subject protection sections.

Recent work on PF-05190457 conducted at NIH and URI during the UH2 phase of the NCATS project

Many pharmaceutical agents variably interact with alcohol. Sometimes these interactions can result in altered drug concentration (PK) because of the influence of alcohol on absorption, distribution, metabolism and elimination. Moreover, alcohol may alter drug effect or pharmacodynamics by changing the affinity of drug receptor binding or altering drug distribution to the site of action (i.e., brain) (Weathermon & Crabb, 1999). PF-05190457 is a substrate for cytochrome P450 (CYP) 3A4 and it is effluxed by P-glycoproteins. Administration of PF-05190457 in subjects with a history of alcohol use and its co-administration with alcohol may therefore yield different pharmacokinetic and/or pharmacodynamic characteristics than those observed in healthy volunteer studies conducted by Pfizer. Therefore, alcohol-drug interactions were studied during the UH2 phase of this grant, as detailed next.

Development of LC-MS/MS Assays

In collaboration with Dr. Akhlaghi's lab at URI, assays for rat brain and rat/human plasma were developed and validated according to FDA guidelines, as recently published (Ghareeb et al., 2015). Assays were used to measure PF-05190457 showing high sensitivity and selectivity.

Preclinical work

In collaboration with Dr. Heilig's lab at NIAAA, we conducted the following preclinical work. First, we tested different doses of the drug and we assessed plasma and brain concentrations using the assays previously developed. We showed brain penetrance after intraperitoneal (i.p.) administration. In addition, analyses of the brain concentration data, together with previous Pfizer data, provided the information that, 3 and 10 mg/kg of i.p. PF-05190457 yielded 50% and 100% receptor occupancy. Then, given that sedation and sleepiness were reported in previous Phase 1 studies conducted by Pfizer, our next goal was to exclude significant sedative effects due to drug-alcohol interaction. In order to do so, we conducted three separate experiments with Wistar rats pretreated with vehicle or PF-05190457 (3 or 10 mg/kg i.p.):

- Locomotor activity (n = 20) 0.5 g/kg (20% v/v) ethanol i.p.
- Locomotor activity (n = 20) 1 g/kg (20% v/v) ethanol i.p.
- Loss-Of-Righting-Reflex (n = 39) 3.5 g/kg (20% v/v) ethanol i.p.

All experiments indicated no significant PF-05190457 / alcohol interactions for the outcomes assessed by these experimental designs.

Clinical work: Protocol 14-AA-0042

Enrollment for protocol 14-AA-0042 was recently completed. This was a single-blind, dose-escalating, placebo-controlled, within-subject study using PF-05190457 (50mg and 100mg b.i.d.) or matched placebo in non-treatment seeking heavy drinking subjects (n = 12 completers). At each visit, subjects received the study drug on Day 1 (twice), Day 2 (twice) and then in the morning of Day 3 (drug dosing phase), after which an alcohol session (drug/alcohol interaction session) was conducted, followed by repeated clinical and research assessments and repeated blood sampling for the PK/PD outcomes. Across the three drug conditions (placebo, PF-05190457 50 mg b.i.d., PF-05190457 100 mg b.i.d.), there were no serious adverse events and the drug was well-tolerated, as patients reached the targeted dose per study design, and none dropped due to study drug-related side effects was reported. During the drug/alcohol interaction session, there were no statistically significant differences in adverse events nor on alcohol-related biphasic effects (sedation and stimulation). During the drug dosing phase, there were no statistical differences in adverse events between the three drug conditions. Analysis of Day 1 EKG vs. Day 3 EKG showed a significant difference in the QTc interval in the two drug conditions vs. placebo; however, this statistically significant difference did not have a clinical significance given that all QTc values always remained within the normal range. Additionally, the statistical significance was mainly driven by a decrease in the QTc interval from Day 1 to Day 3 in the placebo condition. Nonetheless, out of an abundance of caution, the present protocol will have specific safeguards, as detailed in the exclusion criteria and in the criteria for participant withdrawal.

While Protocol 14-AA-0042 was primarily designed as a Phase 1b drug/alcohol interaction study, we also took the advantage of having research participants at the NIH CRC in order to conduct preliminary assessments of the potential role of PF-05190457 on alcohol craving. The main goal of these preliminary assessments was to provide information that may better guide the present protocol. In particular, we found the following:

1. **PF-05190457 did not reduce alcohol craving assessed during drug dosing in the hospital room**

2. **PF-05190457 reduced alcohol priming-elicited craving (Figure 4).**

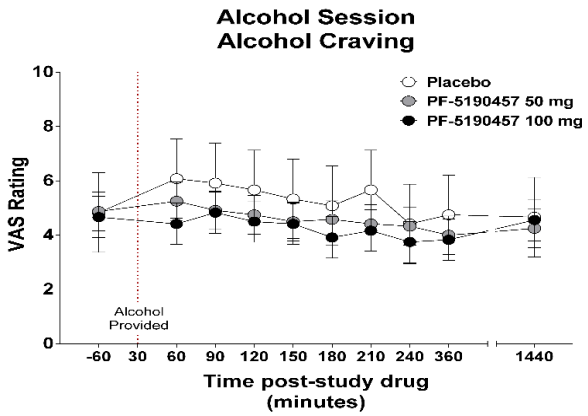


Figure 4. PF-05190457 reduced alcohol priming-elicited craving measured by a Visual Analogue Scale (VAS) (Drug dose*Time: $F(18, 171) = 2.05$, $p = 0.0096$)

3. PF-05190457 reduced alcohol cue-elicited craving in a "bar-like" laboratory (Figure 5)

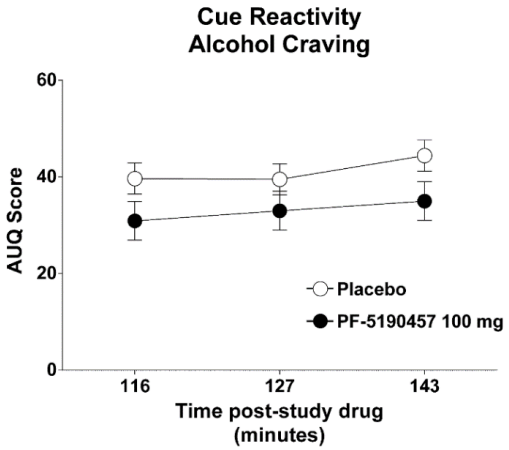


Figure 5. PF-05190457 reduced alcohol cue-elicited craving measured with the Alcohol Urge Questionnaire (Drug: $F(1,13) = 4.45$, $p = 0.05$; dz: 0.78)

These results are very preliminary. Nonetheless they provide additional evidence in agreement with our overall hypothesis and strengthen the rationale for conducting the present protocol. Additionally, the results above are unlikely to be due to non-specific effects of the drug in terms of sedation and sleepiness (Figure 6).

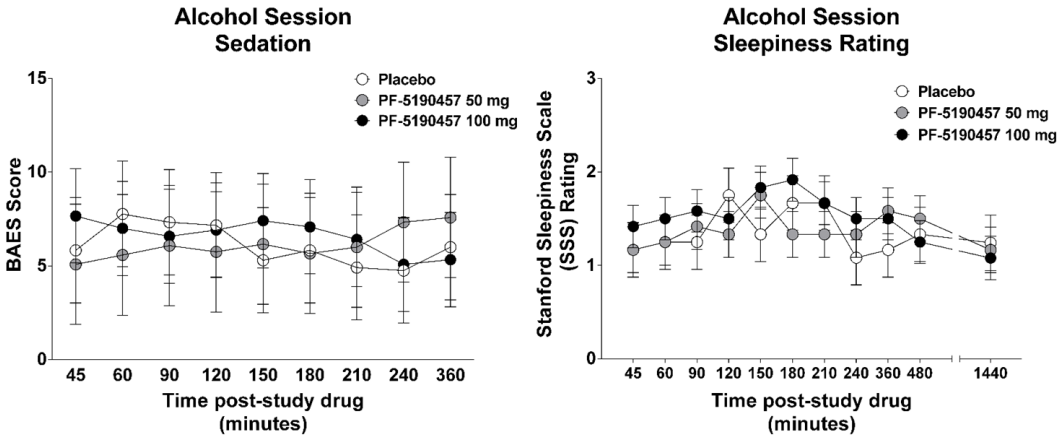


Figure 6. PF-05190457 administration vs. placebo does not result in increased sedation (left) or sleepiness (right) measured with the Biphasic Alcohol Effects Scale (BAES)

Behavioral interventions in AUD and Motivational Interviewing with Video Feedback (MIVF)

Based on the results described above, the overall goal of this protocol is to further investigate PF-05190457 in AUD in order to test if this drug shows an early-signal of efficacy in AUD patients. It is important to keep in mind that most alcoholism treatment is psychosocially oriented and is conducted in the outpatient setting with pharmacotherapy for alcoholism being primarily an adjunct therapy for patients receiving psychosocial interventions (Swift and Leggio, 2009). Compliance with keeping outpatient appointments and compliance with psychosocial interventions that are given in concert with pharmacotherapy is a challenge in addiction treatment as motivation for treatment as well as abstinence often wanes over time. Psychosocial treatments alone are often not effective for everyone and pharmacologic interventions increase the odds for patients to reduce or stop drinking. On the other hand, medications alone may have modest effect sizes when used in real-world clinical settings, therefore they should be considered primarily as adjunct therapy for patients receiving psychosocial interventions (Swift and Leggio, 2009). In conclusion, if this current protocol will be indicative of efficacy for PF-05190457 in AUD, the next large pivotal randomized controlled trial will test this medication in patients who will also receive a behavioral intervention. As such, providing additional scientific evidence for effective behavioral interventions is crucial from a clinical and public health perspective, is in line with the scope of the science proposed in this protocol and is consistent with the overall scope of our laboratory to develop novel treatments for patients suffering from AUD.

Motivational Interviewing with Video Feedback (MIVF) represents an adaptation of an approach that has been shown to shift patients toward behavior change and uses this approach to enhance motivation *over time.* The goal of the proposed Motivational Interviewing with Video Feedback is to explore the effect of video feedback of motivational interviewing sessions in treatment-seeking alcoholic individuals on sustained motivation for abstinence, insight into addiction as well as the ability to reflect about one's relationship with alcohol. Our methodology is supported by theoretical and empirical literature. Motivation for abstinence improves treatment outcomes in recovering alcoholic patients (DiClemente, 1999; Hedeker & Mermelstein, 1996; Wickizer at al., 1994). In general, motivation often peaks at the outset of a person's decision to modify behavior (Orleans, 2000). For alcoholic individuals, motivation fluctuates throughout treatment (Polcin et al., 2004). Muench and colleagues (2006) argue that significant gains have been made in motivating

patients to engage in treatment, but interventions to maintain motivation over time are needed (Muench et al, 2006). In order to sustain motivation, the mental and emotional processes used to prompt abstinence need to be supported over the course of treatment, making the consequences of relapse salient (Miller, 1999). To this end, Bray and Kehle (2001) have developed a video feedback self-modeling technique to film patients in highly motivated states to maintain a behavior change then replay those videos to patients at subsequent points in the treatment in an effort to sustain motivation over time.

One approach to increasing motivation to change is Motivational Interviewing which aspires to enhance the client's intrinsic motivation for change and to maximize commitment to alter behavior and thought patterns. Indeed, a meta-analysis conducted by Rubak et al. (2005) demonstrated motivational interviewing had a significant effect on behavior change in 33 of 42 randomized controlled trials which included patients with alcoholism and drug use disorders. Motivational interviewing enhances motivation, in part, through helping the patient generate *insight* into his or her maladaptive behavior. In alcoholics, insight refers to the ability to understand how the disease affects themselves and those around them. Reduced insight into the consequences of addiction over the course of treatment reduces a patient's readiness to change and long-term sobriety (Blanchard et al., 2003; Vaillant, 1983). Kim et al. (2007) found a correlation between a patient's level of insight and their motivation to abstain from alcohol. Moreover, the same authors also demonstrated that higher levels of insight are associated with longer periods of abstinence in alcohol-dependent patients after one year of discharge from a hospital-based treatment clinic. Evaluating changes in a patient's level of insight, then, will help us predict the authenticity and strength of a patient's motivation to abstain from alcohol.

The capacity for self-reflection or *reflective functioning* promotes insight. Schechter and colleagues (2006) have used video feedback to improve reflective functioning and insight in traumatized mothers with respect to their relationship with their young children. This improvement predicts a shift in mothers' attitudes toward their children that are more age-appropriate and less distorted by their own needs. In alcoholic patients, the greater the patient's capacity for reflective functioning, the greater the likelihood that intervention will alter the patient's intrinsic understanding of their alcoholism and therefore improve their insight and motivation. In addition to maintaining motivation over time, it is important to help recovering alcoholics gain the confidence that they are able to change their behavior. To this end, we will use feed forward self-modeling to help a patient construct an image of success beyond his or her current capacity for achievement. The self-modeling technique combines previously learned behaviors that are components of a new skill, to help an individual perform a new behavior (Dowrick, 2012).

D. **<u>Study Objectives:</u>**

The present protocol represents the UH3 (Phase 2a) of Dr. Leggio's NCATS grant.
We propose to test PF-05190457, a GHSR1a inverse agonist, as a novel treatment for AUD. This will be a Phase 2a, proof-of-concept (early-signal) human laboratory study with PF-05190457 (100mg b.i.d.).

Primary outcome measures:

The primary aim will be to determine whether PF-05190457 reduces alcohol cue-elicited craving assessed in a "bar-like" laboratory. This outcome will be assessed during the inpatient phase of the protocol.

Secondary outcome measures:

The two secondary aims will be to determine:

a. Whether PF-05190457, compared to placebo, reduces brain BOLD response during exposure to alcohol, food and sexually appetitive cues, during a task-based fMRI scan

b. Whether PF-05190457, compared to placebo, reduces food choices in a "virtual buffet" conducted in a virtual reality context.

These outcomes will be assessed during the inpatient phase of the protocol.

Tertiary outcome measures:

The tertiary aim will be to determine whether Motivational Interviewing with Video Feedback (MIVF), compared to standard supportive counselling, will enhance motivation for abstinence in the outpatient setting as well as will increase time to first relapse and reduce alcohol drinking. This outcome will be assessed during the outpatient phase of the protocol. The primary and secondary outcomes of this study will provide guidance on whether a next large randomized controlled Phase 3 trial with PF-05190457 in AUD should be conducted. If that will be the case, the tertiary outcome will provide valuable information on which behavioral intervention may be combined to the pharmacological treatment with PF-05190457 in the future Phase 3 trial. Additionally, this tertiary outcome may provide beneficial information for studies conducted with other medications.

E. **Study design and methods.**

This

Overview. This Phase 2a human laboratory study is aimed to test PF-05190457 100mg b.i.d. for an early-signal of efficacy in treating alcoholism.

Design. A within-subject, counterbalanced, double-blind, placebo-controlled study using PF-05190457, in treatment- and nontreatment seeking individuals with AUD.

Screening. The screening process for this protocol is conducted under the 14-AA-0181 NIAAA screening protocol procedures conducted in the Outpatient Clinic and/or the Inpatient Unit. As routinely done at the NIAAA Intramural Clinical Program, the screening procedures and data collected in the 14-AA-0181 protocol will allow the Investigators to assess the eligibility criteria for this protocol. If suitable, subjects will be offered to participate in this protocol. If subjects agree and sign the consent form, they will be enrolled in the study and start the study procedures.

Study Procedures

This study includes an inpatient phase followed-up by an optional outpatient phase

INPATIENT PHASE

Overview: During the inpatient phase, eligible participants will undergo Stages 1 and 2, where study drug (PF-05190457 or placebo) administration will be counterbalanced. A study drug wash-out window of at least 2 days will take place between Stage 1 and Stage 2. During each Stage, subjects will take the study drug twice a day (b.i.d.) for a maximum of 14 days in order to allow flexibility, maximize feasibility and minimize scheduling problems. At the end of each Stage, the last evening dose of the study medication will not been administered. As part of this study, subjects will be admitted to the inpatient unit, 1SE and will remain as inpatients for the duration of the two Stages and the wash-out window. During the washout period (i.e., when participant is not receiving the investigational study drug), a participant may request an unaccompanied pass to leave the NIH Clinical Center; the PI, MAI or covering physician will evaluate the pass request and determine whether the pass may be granted during the washout period.

The following research procedures (1-3 listed below) will take place once in Stage 1 and once in Stage 2. These procedures will be the same for Stage 1 and Stage 2.

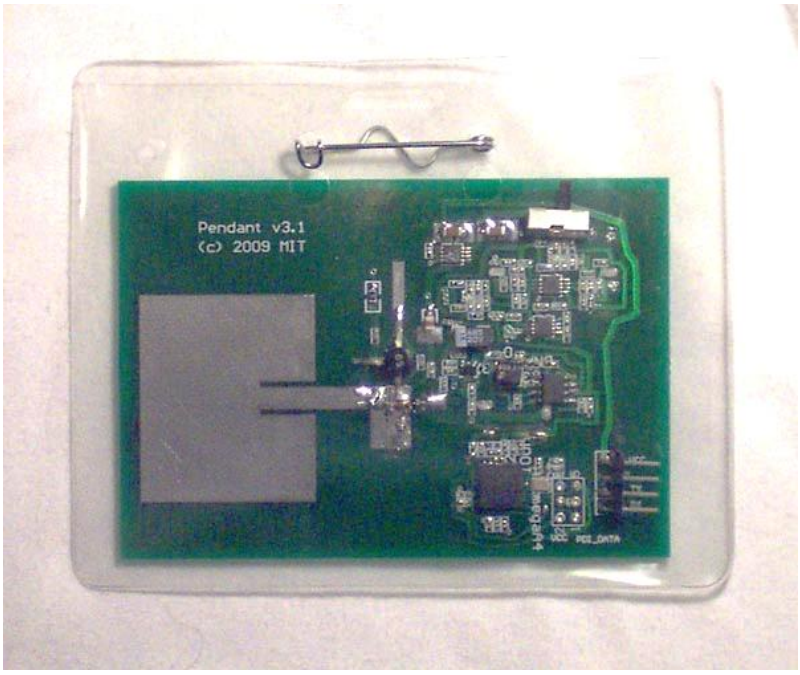
Physiologic Measures: As part of physiologic monitoring in this study, we are using several biosensors (detailed below) provided by Dr. Rich Fletcher at the Media Laboratory, Massachusetts Institute of Technology. Biosensors can provide comprehensive information of one's internal physiological state by measuring multiple physiological parameters continuously over long time scales. Analysis of this composite data can potentially reveal previously unobservable effects in the context of abstinence, medication administration, for example, compared to monitoring singular measures of physiologic data. These sensors are not considered "new investigational medical devices" since the main thing that is new is not the sensors but the packaging/form factors that allow comfortable recording as well as accompanying technology for communicating, analyzing, and visualizing physiological information.

A Material Transfer Agreement is in place to obtain the Biosensors used in this study.

We will use the following sensor:

1) **The MIT bed sensor** is a small flat electromagnetic device which detects small movements. The sensor can be mounted on the wall next to the bed to monitor physical movements during sleep. Alternatively, the device can be placed under the mattress to measure physical movements. When a person is lying directly over the sensor, the device can also be used to measure heart rate and respiration. A photo of the device is shown in Figure 3, below. The device will be placed under the subject's bed on the 1SE unit upon enrollment in the study and will remain there for the duration of their stay on the unit.

Figure 3: Bed motion sensor device packaged in clear plastic holder.



For the purpose of the current study, the bed sensor shall be placed under the mattress, located approximately under the torso area of a person sleeping in the bed. The bed sensor device uses a 5V power adapter that would be plugged into an outlet behind the bed.

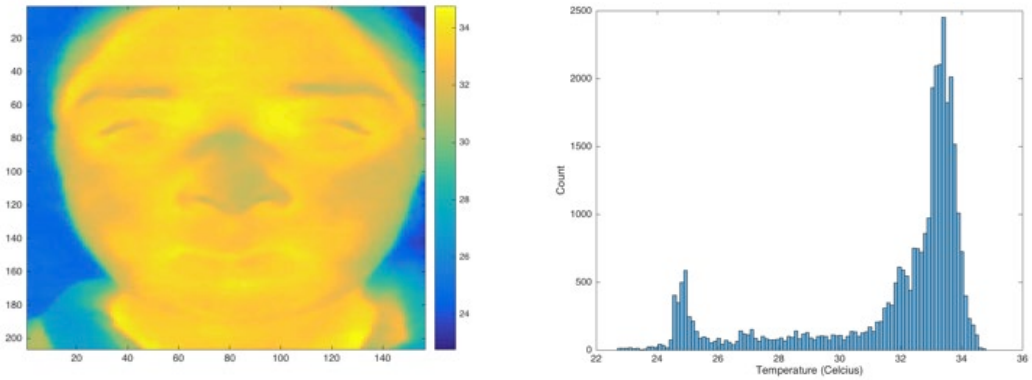
Analysis of the bed sensor data will involve extracting gross motor movements as a function of time. The data extracted is very similar to the data from an accelerometer. These data shall enable a basic analysis of the participant's sleep cycle and the duration of sleep. Depending on the participant's position during sleep, and the relative location of the sensor device, it might also be possible to extract heart rate and breathing rate information during sleep as well.

3. Thermal camera -- the camera will capture a thermal image of the participant's face and will be used during the cue reactivity sessions to detect changes in skin temperature as a consequence of alcohol cue exposure as well as the effect of study drug on these measures. The thermal camera (Seek Thermal, Santa Barbara, CA) is designed to attach to an iPAD. The thermal camera will be installed on an iPAD which will be used to take a thermal facial image at baseline and at several time points during the cue reactivity and alcohol administration procedures. The Apple iPad tablet will be mounted on a wall in the bar room.

Figure 4: Seek Thermal Camera

Dr. Fletcher at MIT has developed software tools that are used to analyze the images in order to calculate thermal gradients and thermal patterns on the face. The camera takes images that are 206 by 156 thermal pixels. The software contains an algorithm to isolate the face, and then samples thermal data from different parts of the face and generates histograms showing the normalized temperature differences at different points on the face.

Figure 1. (left) Sample thermal image, and (right) sample histogram.



1. Cue-Reactivity (CR) procedure

This procedure will be similar to that used in our previous studies, including 14-AA-0042 and 13-AA-0040. The CR procedure is performed in a bar-like laboratory. During the CR procedure, participants are exposed to visual, tactile, olfactory, and proprioceptive stimuli associated with the beverage. Participants first will undergo a 3-minute relaxation period (‘‘please sit quietly and do nothing’’) to collect pre-CR levels of urge and physiological arousal (this first relaxation period may take place outside in a clinical testing room or inside the bar-like laboratory). There will be a tray containing a glass half full of water and a commercially labeled bottle of water located in the room. An audiotape will instruct the participant to sniff the glass of water when s/he will hear high tones and stop sniffing when s/he will hear low tones. This procedure will include thirteen 5-second olfactory exposures during each 3-minute trial, with variable intervals between each exposure. The water

trial provides a controlled baseline that controls for all aspects of stimuli and movement except for the nature of the beverage. After that, another 3-minute relaxation period will follow. Next, participants will undergo a similar procedure with personalized food cues; this will be used given that our preliminary data from 14-AA-0042 show an effect of the study medication on food craving assessed during the CR. Finally, two 3-minute alcohol cue exposure trials that will be identical to the water trial except the glass of water will be replaced with their preferred alcohol beverage and the bottle of water will be replaced with the appropriate commercially-labeled alcohol bottle. Two alcohol trials will be conducted to gain a stable estimate of participants' reactions to alcohol cues and because two exposures have proven most sensitive to differential effects in previous studies (trials will be presented in the same order for all participants because of known carryover effects). After every 3 minutes of beverage exposure, participants will rate their urge to drink alcohol by completing the Alcohol Urge Questionnaire (AUQ) (Bohn et al. 1995) (Appendix 9) . The term urge to drink is explained to the subject as "want, desire, craving, thirst for or wish to drink". The AUQ will be complemented by the Alcohol Attention Scale (AAS), which consists of two 10-point Likert-type scales assessing attention to the sight and smell of water and alcohol cues.

2. Functional Magnetic resonance imaging (fMRI) [for those who have no exclusion criteria for fMRI scanning or due to scheduling limitations, e.g. scanner shutdown]

During scanning, subjects will view alternating blocks of pictures of the following stimuli: 1) alcohol cues, 2) food cues and 3) sexually attractive cues. Food and alcohol cues will be from online and in-house collections. We picked four images of males and four images of females that are most highly rated for positive valence in the Erotic/Romance category of the International Affective Picture System (IAPS), based on a previous study by Ely et al. (2015). Control cues for this task will be taken from the NimStim picture set. The use of sexually attractive cues (other than alcohol and food) is an exploratory additional outcome we would like to investigate, in a preliminary manner, based on previous preclinical data indicating that ghrelin signaling within the tegmental areas is required for sexual behavior in sexually naïve male mice (Prieto-Garcia et al., 2015; Egecioglu et al., 2015). The order of all tasks, food/alcohol/sexual as well as the cognitive measures (described below), will be randomized. Emerging research also links ghrelin signaling to neuromodulation, neuroprotection, memory and learning processes (for review, see Gahete et al., 2011), therefore we will also conduct two cognitive measures, i.e., Stroop and inhibitory Control:

a) Stroop: Subjects are presented with various words written in different text colors. Subjects are asked to identify the color of the text. This is a measure of both attention and executive function. (time to complete: ~ 5 minutes). Inhibitory Control: assessed through a GO-NOGO task. Stimuli consist of an alternating series of X's and Y's presented at one per second. Participants are told to press a button for each target stimulus while the stimulus is still present on the screen. Successful performance on this task requires prepotent behaviors to be inhibited. Note: The Go-NoGo task is approximately 25 minutes duration and not the primary imaging task we are investigating. Therefore, in order to avoid fatigue and discomfort that could impinge on primary task outcome (food/alcohol/sexual cues) we will administer this task last.

The functional scans, will be acquired while the subjects are at rest (to conduct connectivity analyses) and while they participate in the tasks described above. Imaging will take place at the NIH Clinical Center. An initial structural scan will be collected on each subject for later co-registration of functional images. The structural scan will last approximately 10 minutes. Functional scans will be acquired using a T2*-EPIRT sequence that measures changes in BOLD contrast. Visual stimuli will be projected onto an opaque screen at the foot of the imaging machine via a liquid crystal diode projector. Subjects will be able to view the images via a mirror placed above their head in the scanner. Subjects will remain in contact with the experimenters via an intercom while they perform the tasks.

3. Food choices in the Immersive Virtual Environment Test Unit (IVETU)

As reviewed by Volkow et al. (2013), there is increasing evidence that disruption of energy homeostasis can affect the reward circuitry and that overconsumption of rewarding food can lead to changes in the reward circuitry resulting in

compulsive food intake akin to the phenotype seen with addiction. Consistent with this concept, several lines of experimental research demonstrate significant commonalities between the neural substrates underlying addiction and at least some forms of obesity (Volkow et al., 2013). Consistent with this body of literature, it is conceivable that a medication effective in reducing alcohol craving and drinking, may also have effects in reducing appetite and food intake. This is the case, for example, of the FDA-approved naltrexone and of topiramate (for review, see: Leggio et al., 2011). Notably, our preliminary work (protocol 14-AA-0042) indicated that PF-05190457 reduced food cue-elicited craving in a "bar-like" laboratory. Albeit interesting, this preliminary finding needs to be further investigated. Therefore, we will explore the potential role of PF-05190457 administration on food-related behaviors. To achieve this goal, we will use the National Human Genome Research Institute (NHGRI) Immersive Virtual Environment Test Unit (IVETU) located on OP6 in the NIH Clinical Center in order to assess the potential effects of PF-05190457, compared to placebo, in a "virtual buffet." The "virtual buffet" in the NHGRI IVETU has already been tested by Dr. Persky's group in other IRB-approved clinical protocols at NHGRI (e.g., Bouhlal et al., 2015). This procedure permits us to investigate, in a well-controlled setting, direct observations of patients' food selection behaviors. Participants will be given verbal instructions and will have a training session on how to use the "virtual buffet". Participants will be instructed to choose foods and a beverage from a hypothetical buffet. In order to perform the virtual reality task, participants will wear a head mounted display connected to computer equipment and will use a pointing device to select the desired types and amounts of food and beverage. Included on the "Virtual Buffet" will be foods and beverages representative of those typically found at buffet restaurants and comprising a range of nutrient profiles and calorie densities. Consistent with the procedures already described in Bouhlal et al. (2015), participants will be instructed to choose as many and as much of the virtual food and beverages during one trip to the buffet as they would normally choose. Once the virtual plate is full, participants will have an opportunity to go back to the buffet and select additional virtual food. The buffet will contain at least two options for each food category that would typically be present at lunch (main dish, vegetable, fruit, starch, dessert, and beverages). Foods will be categorized as "Go" (the most healthy options), "Slow" (less healthy options) and "Whoa" (relatively unhealthy options), as described before (Bouhlal et al., 2015). Participants will indicate their food choice by using a pointing device and then will select the amount of food that will go on the plate (e.g., one spoonful, one piece). Following food selection, participants will be able to choose from several possible beverages. The virtual scenario will end when participants indicate they are finished selecting food and drink. During all phases of the scenario, we will digitally record participants' location in the room, the direction of participants' gaze, duration of the session, and all details regarding food selection. The patient's food choice behavior will be assessed by calculating the total calories selected including foods and the beverage. Calorie content will be assessed by using the volume of the virtual food chosen by participants to calculate the appropriate number of calories based on information contained in food nutrient databases. Food-related outcomes will be analyzed, including calorie content of food and drink chosen, patterns of engagement with the food, order of food selection, time spent in the buffet, portion sizes selected, distribution of different food types on the plate, total calorie count of selected food with and without beverages, proportion of high calorie foods, sweetened beverage selection, spatial measurements, and food choice process. At the end of the procedure, participants will also fill out questionnaires to assess participant's experiences in the virtual environment (see Appendix section).

4. Additional data that will be collected during the inpatient phase:

During the study, the following data will also be collected:

- Alcohol Urge Questionnaire (AUQ)
- Food craving using the General Food-Cravings Questionnaire (GFCQ) Trait (at baseline of Stage 1) and State
- Affective mood states using the Profile of Mood States (POMS)
- Blood samples for pharmacokinetic and research use (see "Use of Samples" section G below for details)
  These data will be collected at the beginning of each Stage (before the first study drug administration) and at the end of each Stage. These data outcomes will also be collected twice (pre- and post-procedure) during each

of the procedures described above, i.e., CR procedure, fMRI scan day whether subject has fMRI or not and IVETU procedure.

- Blood samples for pharmacokinetic and research use will be taken daily (see "Use of Samples" section G below for details); additional blood samples will be taken post-testing for the CR, fMRI, and IVETU tasks
- Waist circumference will be assessed twice during each Stage (first and last day of each Stage) in order to calculate the body shape index (BSI), which is, compared to BMI, a better indicator of health risks related to excess weight.
- The Snaith Hamilton Pleasure Scale (SHAPS) (at baseline, end of Stages 1 and 2) to assess potential effects of the study drug on hedonic capacity.

5. Subject characterizations: The following are characterization measures of various behaviors and preferences administered either in questionnaire or task form. With the exception of smoking, all of these will be done once at the beginning of the study before study drug is administered (please see Appendix 2 – Table Outline for the Inpatient Phase for timing of these procedures).

a) Sweet Preference Test. There is preclinical and clinical evidence linking the consumption of sweets to alcohol intake in both animals and humans (Leggio et al., 2011). We will test the possible effects of individuals' sweet preference characteristic on our study outcomes. Prior to administration, a visual examination of the sucrose solution for any particulate matter will be conducted by a clinician, proceeding only if no particulate matter is identified. Each subject will be instructed to sip, swish around his/her mouth and then spit out five different concentrations of a sweet solution (0.05, 0.10, 0.21, 0.42 and 0.83 M) each of which will be presented five times in a pseudorandom order for a total of 25 tastings. After each tasting, participants will rate the intensity and pleasurableness of each tasting using a 200-mm analog scale. Then, participants will rinse their mouths with distilled water before proceeding to the next solution. Subjects will be characterized as sweet-liker or sweet-disliker based upon their hedonic response to the various sucrose concentrations. To be categorized as a sweet-liker, a subject must rate the highest concentration of sucrose (0.83 M) as the most pleasurable. Sweet-dislikers could prefer any of the other four concentrations. As part of this test, subject will also fill out the Sweet Preference Questionnaire (see Appendix 7 and corresponding SOP for reference in Appendix 12).

b) Brief Addictive Behavior Social Density Assessment (BASDA). The BASDA is a self-reported assessment where participants self-report on their closest associates' drinking behaviors (Bollinger et al., 2012). Participants are not asked to provide the names of their closest biological and non-biological associates. The goal is to explore the social milieu that usually surrounds the participant and to see if this information might help to predict craving behavior in our human laboratory setting.

c) Behavioral approach system (BAS)/behavioral inhibition system (BIS) scale. The BAS/BIS scale was developed to measure individual differences in the sensitivity of the BIS and BAS (Carver & White, 1994). The scale is divided into 4 subscales: BAS Drive, BAS Fun Seeking, BAS Reward Responsiveness, and BIS. Much research suggests these systems play an important role in vulnerability to addiction. The individual differences in reward sensitivity measured by these scales are especially relevant to addiction when examining the role of the DA reward system. The goal of this assessment in the current study is to further explore how BAS and BIS personality traits relate to alcohol craving and the activation of reward circuitry.

d) Virtual Buffet (IVETU) Questionnaires (Appendix 7): The following questionnaires are assessments of individuals' attitudes and cognitions relating to eating and weight that have been linked with behavior change. These characterization measures may help to explain variations in subject behavior in aspects of the trial (e.g., the virtual buffet, outpatient behavioral intervention).

1. Perceived Family History: weight and alcohol problems

2. Implicit Theories of Weight, Alcohol Use and Eating (Burnette, 2010)
3. Causal Beliefs Scale for Alcohol Dependence and Casual Beliefs Scale for Body Weight (Link et al., 1999; Moss-Morris et al., 2002)
4. Perceptions of Personal Control and Treatment Control (Broadbent et al., 2006)
5. Self-Stigma and Alcohol Dependence (Schomerus et al., 2011)
6. Perceptions of passing down propensity for disease (Persky et al., 2015).

f) Smoking. The high co-morbidity between alcohol and nicotine dependence reflects a strong connection between alcoholism and smoking (Littleton et al., 2007). We will assess the severity of nicotine dependence with the Fagerström Test for Nicotine Dependence (FTND; Heatherton et al., 1991), cigarettes smoked before and then during the duration of the study (via the TLFB) and Breath CO levels, for possible exploratory analyses, which might guide future alcohol/smoking studies. At the NIH Clinical Research Center, inpatients can smoke in a specific smoking area whose access is controlled by our nursing staff. Therefore, we will be able to collect the precise number of cigarettes smoked during the inpatient stay. Furthermore, when experimental procedures will take place, smoking breaks will be standardized across and between subjects. Finally, during each Stage, we will perform a smoking topography procedure to assess smoking behaviors such as smoking intensity (e.g., number of puffs, inter-puff intervals, peak puff velocity), while subjects smoke their first cigarette of the day on specified days during the study in the NIH CRC smoking area – this will help us to pilot a smoking topography device that we have recently purchased and would like to use in future studies.

g) Genetics. A single whole blood sample (~2 mL) from each subject will be shipped to Dr. Akhlaghi from URI where it will be stored and DNA analyzed for polymorphisms of genes involved in PF-05190457 metabolism and transport, alcohol metabolism and ghrelin activity.

6. Nutrition

Participants will receive standardized meals developed in collaboration with the NIH Nutrition Department to control and monitor caloric intake within and across participants. Food-related data, including % of meal eaten, will be recorded. Participants may receive a regular diet during the washout period.

7. Study Medication Check

There is a possibility that subjects and/or Investigators might identify if they received the PF-05190457 compound or placebo. Therefore, at the end of each Stage, we will ask subjects and study physician which they believed they received. This information will be recorded.

8. Measurements for safety monitoring

The following safety measures will be performed repeatedly as per below (and also as needed, as the clinical situation dictates):

- Vital signs, weight and BrAC (every day during each Stage, prior to study drug administration)
- Check for potential adverse events (every day during each Stage)
- Blood glucose via finger stick (3 times during each Stage)
- Stanford Sleepiness Scale (3 times during each Stage)
- EKG, electrolytes and liver and kidney function tests: at the beginning of Stage 1 before administration of the study drug and again at the end of Stage 1, and then twice during Stage 2 (before first drug administration and at the end of Stage 2)

9. Additional Considerations

Medication Dose Justification. The dose of 100 mg b.i.d. was the highest dose used in the previous study (14-AA-0042) and was the dose that led to a reduction in cue-induced craving (Figure 5), the primary aim for this study. The same dose of 100 mg b.i.d. was previously used in the last Phase I study (B3301002; clinicaltrials.gov: NCT01372163) conducted by the manufacturer (Pfizer).

Medication Compliance. During the study, the drug will be administered at the NIH Clinical Center, which will assure 100% compliance with the study medication.

Missing data. While missing data will be minimized, we do expect that we will not always be able to collect all planned data (an example of a scenario is missing blood samples due to problems with the cannula, hemolysis, and other technical problems). Nonetheless, every effort is made to minimize these unforeseen problems with equipment or study procedures. If one should occur, we will ask the participant to repeat a specific experimental procedure (if allowed by the overall study schedule, if it is feasible and if the participant agrees) in order to avoid having to discard other usable data from a participant. Subjects may receive additional compensation if experimental sessions are repeated, consistent with the NIDA/NIAAA Remuneration Policy.

Data sharing with other protocols. Data obtained under this protocol and the NIAAA screening 14-AA-0181 protocol as well as with Protocol 14-AA-0066, Behavioral and Functional MRI Task Development, Implementation and Testing may be shared and combined for analysis. Both protocols, 14-AA-0181 and 14-AA-0066 protocols are protected by a Certificate of Confidentiality. This will also allow to avoid repeating assessments that are scheduled in both protocols during the same period of time, therefore avoiding duplication ad minimizing participants' fatigue.

This protocol does not meet criteria for genomic data sharing.

OUTPATIENT PHASE

11. Follow-up Visits and Behavioral Intervention

After the inpatient phase is completed and the patient is discharged, s/he will be scheduled to come back to the NIH for up to 8 optional outpatient follow-up visits. The first outpatient visit will be approximately a week after inpatient discharge, with flexibility based on Outpatient Clinic and patient's schedule. During these outpatient visits, BrAC, vital signs and weight will be assessed. At each visit, the patient's alcohol and drug use will also be assessed using the Timeline Follow-Back (TLFB). If BrAC is positive, subjects will wait until BrAc is below 0.00 (g/dl) to begin session or will be rescheduled and offered cab transportation home unless a licensed driver accompanied the patient and will be providing transportation. During these outpatient visits, male patients will also be reminded about necessity to refrain from sexual activity for the 28 days following the last dose of study medication (details in the inclusion criteria Section F-b and in the consent form).

During the follow-up visits, patients will receive a behavioral intervention. We propose to use video feedback of motivational interviewing sessions, and, as a comparison intervention, supportive counseling. The motivational interviewing with video feedback incorporates principles from feed forward learning strategies, video feedback interventions and motivational interviewing to promote sustained motivation for abstinence, insight into addiction and confidence to change behavior.

11.1 Measures (see also Appendix)

Motivation: The Stages of Change Readiness and Treatment Eagerness Scale (SOCRATES) (Miller and Tonigan, 1996) measures whether or not patients recognize drinking problems, ambivalence or uncertainty about drinking, and whether or not the patient is currently taking steps to change. It is a measure of overall motivation for abstaining from alcohol. Situational Motivation Scale (SIMS) (Guay et al., 2000): evaluates the specific type of motivation prompting behavioral change – it differentiates between four different types of motivation: intrinsic, identified regulation, external regulation, and amotivation. The questionnaire uses the following definitions of each motivational type:

- Intrinsic motivation: behaviors that are engaged in for their own sake, in other words, for the pleasure and satisfaction derived from performing them.
- Identified regulation: behaviors the patient recognizes as beneficial, but ones that she pursues because of its perceived outcome, rather than because she finds the behaviors inherently satisfying
- External regulation: behavior patients feel forced into because it regulates rewards or helps them avoid negative consequences
- Amotivation: behaviors pursued with no sense of purpose or expectation of rewards

Insight: The Hanil Alcohol Insight Scale (HAIS) (Kim et al., 1998) is a well validated, 20-item questionnaire used to objectively evaluate a patient's insight into their alcoholism. *Reflective Functioning:* Beck Cognitive Insight Scale (modified) (Beck et al., 2004) measures "cognitive insight," or a person's capacity to achieve insight into a specific problem or issue. The 15 item scale evaluates capacity for insight through questions that reflect a patient's self-certainty and self-reflectiveness and we have modified this scale to focus on alcohol use. The Mindful Attention Awareness Scale (Brown and Ryan, 2003) is a 15 item scale that measures dispositional mindfulness and the Curiosity and Exploration Inventory-II (Kashdan et al., 2009) is a 10 item scale that measures the tendency to seek and embrace new knowledge and experiences. The University Rhode Island Change Assessment Scale (URICA) (DiClemente & Hughes, 1990) is a 32-item self-report measure that includes 4 subscales measuring the stages of change with respect to treatment for substance use disorders: Precontemplation, Contemplation, Action, and Maintenance.

Shift in attributions question (modified from Schechter et al. (2006)) measures shifts in attitudes toward alcohol: "*Tell me 5 words or short phrases (adjectives) that describe how you think alcohol affects you*."

We hypothesize that video feedback in conjunction with motivational interviewing and self-modeling, then, has the potential to help patients to shift motivations and behaviors towards alcohol, as well as maintain abstinence or reduce alcohol drinking, over time.

11.2 Overview of the Procedure

During this outpatient phase, patients will come into the NIAAA Outpatient Clinic at the NIH Clinical Center for 8 visits and there will be two phases: 4 visits of motivational interviewing sessions with video feedback and 4 visits of supportive counseling, using a crossover design with the order of the two phases counterbalanced across participants. All counseling sessions will be conducted by a physician, nurse practitioner, nurse or masters level counselor.

Motivational Interviewing with Video feedback Phase

Motivational interviewing sessions will be conducted at the initial visit and at alternating visits thereafter with video feedback of these sessions during the intervening weeks. During the videotaped motivational interviewing session, the patient and clinician will discuss in progression the following topics:

- Reasons for abstinence
- Triggers and the patient's reactions (physical, mental) to them
- Reasons for abstinence having just discussed their triggers
- Description of a time when the patient refused alcohol
- Reasons for abstinence having just discussed a time when they had successfully refused alcohol

We start the conversation with a discussion about the reasons for abstinence to gain insight into why they want to stop drinking prior to making them conscious of how they react to alcohol and how they have tried to avoid it in the present or the past. The second portion of the discussion is a dialogue about the patient's triggers and responses to them. We included this question to instigate a conversation that will help us understand how a patient's motivation shifts when presented with triggers. Following a conversation about the triggers, we return to a discussion about the patient's motivation for abstinence. We chose to include a discussion about a patient's drive to abstain from alcohol after discussing triggers to see whether or not conversations about experiences with alcohol, or people and objects that remind them of alcohol, causes any variation in the way patients discuss their motivation. The fourth portion of our conversation on motivation for abstinence will discuss how patients have successfully coped with triggers and situations where alcohol was present. This portion of the conversation serves two purposes: to help patients understand how pre-existing and developed behaviors have laid the foundation for successful abstinence, and to give them a chance to reflect on the experience of refusing alcohol. Lastly, we end with a discussion of a patient's motivation for abstinence, to evaluate the impact discussions of successful abstinence when faced with alcohol or alcohol-related stimuli may have on a patient's reasons for motivation or self-reported motivation levels. The session should take 30-45 minutes to complete. Between video-taping and motivational interviewing sessions, the clinician will review the video-tape for clips that represent positive motivations, negative motivations, strong reactions to triggers, and opportunities to present models of successful behavior for the patient to try and emulate.

Visits 1 and 3: At Visit 1, the subject will complete the following questionnaires at baseline:

- Motivation to change (SOCRATES) (Miller & Tonigan, 1996)
- Situational Motivation Scale (SIMS) (Guay et al., 2000)
- Hanil Alcohol Insight Scale (HAIS) (Kim et al., 1998)
- Beck Cognitive Insight Scale (Beck et al., 2004), modified
- Attributions towards alcohol (modified WMCI question) (Schechter et al., 2006)
- Mindful Attention Awareness Scale (Brown and Ryan, 2003)
- Curiosity and Exploration Inventory-II (Kashdan et al., 2009)
- University Rhode Island Change Assessment Scale (URICA) (DiClemente & Hughes, 1990)

- Meta-cognition tasks: Meta-cognition, an individual's ability to comprehend and be mindful of their thought process, is thought to be disrupted in individuals that abuse substances (Goldstein et al., 2009). One task will measure an individual's sense of agency (the amount an individual feels they are the cause of actions around them).

The other task will measure individual's perceptual meta-cognition abilities, or how well they believe they can discriminate between two stimuli.

1. Agency Task: The agency task was designed to assess individual's sense of control (Metcalfe et al., 2007). Individuals will use a computer mouse to "catch" falling stimuli (Xs or Os) that are randomly distributed across the screen by moving a small box across a horizontal track. Individuals will then be assessed on their subjective feelings of control over the small box and performance on the task. Turbulence of the mouse, speed of the scroll, and density of targeted stimuli will vary across the 24 trials conducted. This allows us to collect both subjective (self-reported control and performance) and objective (turbulence of the mouse and actual performance) to compare an individual's ability to think about their performance and agency.
2. Flemings Dots Task: The Fleming Dots task was developed to assess perceptive meta-cognition. Two circles will appear on the computer screen containing varied number of dots. After the two circles disappear, individuals undergo a forced choice paradigm, in which they choose the circle that contained the most dots. The difference in dots between the two circles is titrated so that after two continuous correct answers the difference in the number of dots between the two circles decreases, and after one incorrect answer the difference in the number of dots increases. This is done so that the difficulty of the task is equal between participants (Fleming et al., 2010). Individuals are then asked to rate how confident they feel about their answer.

Then the patient will undergo a motivational interviewing session as described above. For Visit 3, a videotaped motivational interviewing session only will be done.

Visits 2 and 4: During these visits, the patient will view the video of the previous week's videotaped session. Clinicians who run motivational interviewing video feedback sessions with patients should pre-select four thirty second clips to highlight while the patient and clinician watch the entire video clip from the previous week. The clinician should select video clips that represent the following:

- An "optimal" moment – a moment when the clinician believes the patient discusses their reasons for motivation most authentically.
- A moment of stress – a moment when the patient demonstrated a stress or emotional reaction to discussions of triggers or encounters with alcohol
- A moment of successful coping – a moment when the patient discusses successful restraint in using alcohol when presented with triggers or situations related to alcohol.
- A "suboptimal" moment – a moment where the clinician believes the patient's reasons for abstinence sound superficial and disingenuous.

We have chosen to identify optimal, stressful, successful coping, and suboptimal moments because previous studies have shown the importance of making patients aware of the thought processes and patterns that make them susceptible to relapse. Allsop & Saunders (1991) demonstrated that "the processes involved in relapse are identical to those involved in the making and breaking of any resolution to change any behavior". Thus, the optimal moment and moment of successful coping help the patient recognize the appropriate attitudes and decision making processes that will help them abstain from alcohol over time. Identifying the moments of stress and disingenuous reasons for motivation, likewise, increase awareness of situations and thought processes that may make the patient more prone to relapse. These feedback sessions will also be videotaped.

The videotapes will also be coded for verbal and nonverbal behavior with respect to motivation for change. A Reliance Agreement has been put in place with Montclair State University, where AI Dr. Amrhein will assist in developing a rating for

assessing the videotaped motivational interviewing sessions and feedback sessions that will be coded for verbal and nonverbal behavior with respect to motivation for change.

After the feedback session, patients will fill out the following questionnaires to assess changes in motivation, insight, cognitive insight, and attributions towards alcohol at intervals throughout the experiment: SOCRATES, SIMS, HAIS, modified Beck Cognitive Insight Scale (modified), the modified WMCI question, the Mindful Attention Awareness Scale, the Curiosity and Exploration Inventory-II, the URICA and Metacognition Tasks: Agency Task and the Flemings Dots Task.

Supportive Counseling Phase

The 4 visits of supportive therapy will consist of outpatient visits with supportive counseling sessions lasting approximately the same duration as the motivational interviewing sessions. In addition, the questionnaires: SOCRATES, SIMS, HAIS, modified Beck Cognitive Insight Scale, Mindful Attention Awareness Scale, the Curiosity and Exploration Inventory-II, URICA and the Meta-cognition tasks: Agency Task and Flemings Dots Task will be completed after sessions on Visits 2 and 4 of this phase.

F. **Inclusion and Exclusion Criteria**

a. Description of study population

This study will enroll individuals with AUD who are treatment- or non-treatment-seeking. The following inclusion/exclusion criteria will be assessed via the NIAAA Screening Protocol 14-AA-0181:

b. Inclusion criteria

1. Male or female individuals 18-70 years old (inclusive)
2. Current Alcohol Use Disorder (AUD) by DSM-5 criteria based on the SCID
3. Most recent urine drug test for illegal drugs of abuse is negative
4. Most recent Clinical Institute Withdrawal Assessment for Alcohol – revised (CIWA-Ar) score is ≤ 8
5. Heart rate ≤ 100 on two separate measurements, both assessed after CIWA-Ar score is ≤ 8
6. Female subjects must be of non-childbearing potential as defined by at least one of the following criteria:

   a) Females 45 –70 years old, who are menopausal, defined as follow:

   i) Females who are between 45 – 55 years old: they will be considered menopausal if they satisfy all the following three requirements during screening: 1) they are in amenorrhea, defined as absence of menstruation for the previous 12 months; 2) they have a negative urine pregnancy test; and 3) they have a serum FSH level within the laboratory's reference range for postmenopausal females.

   ii) Females who are between 56 – 70 years old: they will be considered menopausal if they are in amenorrhea, defined as absence of menstruation for the previous 12 months before screening.

   OR

   b) Females 21-70 years old, who have a documented hysterectomy and/or bilateral oophorectomy.

All other female subjects (including females with tubal ligations and females that do NOT have a documented hysterectomy) will be considered to be of childbearing potential.

7. Male subjects must use one of the following methods of contraception from the first dose of study medication and until 28 days after dosing (given that it is unknown whether the effects of this drug can cause birth defects):

   a. Abstinence.

   b. A condom AND one of the following:
   - Vasectomy for more than 6 months.
   - Female partner who meets one of the following conditions:
     a) Has had a tubal ligation, hysterectomy, or bilateral oophorectomy;
     b) Is post-menopausal;
     c) Uses one of the following forms of contraception:
        - Copper or hormonal containing IUD;
        - Spermicidal foam/gel/film/cream/suppository;
        - Diaphragm with spermicide;
        - Oral contraceptive;
        - Injectable progesterone;
        - Subdermal implant.

c. Exclusion criteria

1. Lifetime clinical diagnosis of schizophrenia or bipolar disorder

2. EKG with QTc > 450 msec as determined by the Fridericia formula

3. BMI < 18.5 kg/m^2 or anorexia

4. BMI ≥ 40 kg/m^2

5. History of epilepsy and/or seizures
   NOTE: individuals who have a history of alcohol withdrawal seizures may be in the study as long as they have been abstinent from alcohol for at least 2 weeks prior to consent and during that period of abstinence, there were no seizure episodes (otherwise, participant remains not eligible).

6. Most recent blood tests show creatinine ≥ 2 mg/dL, AST or ALT > 3x the upper normal limit, hemoglobin <10.5 g/dl

7. Subjects who have diabetes and/or are treated with any drug with glucose lowering properties such as sulfonylurea, insulin, metformin, thiazolidinediones (TZD), Dipeptidyl peptidase-4 (DPP4) inhibitors, or Glucagon-like peptide-1(GLP-1)agonists (due to the glucose-lowering properties of PF-05190457 observed in healthy volunteers)

8. Exclusionary Medications:

   A.
   Naltrexone, acamprosate, alcohol dehydrogenase inhibitors, topiramate, gabapentin, ondansetron, benzodiazepines, and alpha-1 blockers, baclofen, drugs that are known to prolong the QTc interval and barbiturates

as well as hormone replacement therapy; medications and dietary/herbal supplements (like St. John's wort) that interact with Cytochrome P450 3A4. Patients who take these medications may be enrolled in the study only if the potentially interacting medication has been stopped for a period of at least 5 half-lives of the interacting medication before PF-05190457 administration. Patients who take these medications on an as needed (PRN) schedule or take the medication as a one-time dose as part of a medical procedure or a diagnostic test, for example, may not have to wait the 5 half-lives' period of time before enrollment; this will be evaluated on a case by case basis by the MAI and/or PI, based on the specific pharmacological properties of the medication.

9. Unable to pass a finger rub hearing test

10. Vision is unable to be corrected to (Snellen) 20/100

11. Clinically-significant history of motion or car sickness, or history of vestibular disorders

12. Any other reason or clinical condition for which the PI or the MAI will consider unsafe for a possible participant to participate in this study

Exclusion Criteria for fMRI only:

1. Have contraindications for brain fMRI, as determined by the NIAAA MRI Safety screening form (conducted under the 14-AA-0181 Screening Protocol)

2. Colorblindness (this would prevent subject from completing the Stroop task) using the Ishihara Test for Color Deficiency, Concise Edition, 2014.

G. **Collection and Storage of Human Specimens or Data**

1. Use of samples

Blood samples will be used for:

a) Pharmacokinetic use: samples will be used to analyze PF-05190457 plasma concentrations, its known metabolites, and conduct PK/PD analyses (analyses conducted at Dr. Akhlaghi's URI Lab) as per fully executed MTA.
b) Research use: samples will be used for future analyses (performed either in our own lab at NIH or at Dr. Akhlaghi's URI Lab) of blood ghrelin levels, as well as other feeding-related peptides (e.g., leptin, GLP-1, amylin, GIP, pancreatic polypeptide, PYY, GH, insulin, etc.), stress-related hormones (e.g., ACTH, cortisol, etc.) and/or inflammatory markers (e.g., cytokines, etc.) that may be analyzed for exploratory analyses.
c) Clinical use: samples will be used to analyze clinical labs via the NIH Clinical Center Department of Laboratory Medicine (e.g., glucose, AST, ALT, GGT, bilirubin, Creatinine, and electrolytes).

2. Sample Storage

Data will be stored on NIH data servers, under the management of the ORIT, NIAAA and under the management of the NIDA IRP Biomedical Informatics Section (BIS). The existence and types of information contained in the data management system have been publicly reported as required by the FOIA.

Blood not processed via the Department of Laboratory Medicine at the Clinical Center will be processed and stored in -80 °C freezers. All biological specimens obtained under this protocol will be stored in coded form (protocol plus subject number) in freezers located in the access-controlled laboratory area of NIDA. Additional samples will be shipped to URI, Dr. Akhlaghi's lab. All results generated in Dr. Akhlaghi's lab for this project will be sent to the PI and his team at NIH.

Storage of video tapes: Video tapes will be stored on a password protected computer operated by an approved research team member under supervision of the PI. A copy of videotapes will be sent to Dr. Amrhein's registered email address through NIH secure email file transfer. All tape recordings and copies made that are collected under this protocol will be destroyed no later than 1 year following study completion.

Biosensor Data

Data from biosensors will be sent electronically to Dr. Fletcher's laboratory at MIT where it will be analyzed.

Data being analyzed will be identified by participant codes and identifying information will be removed. Each participant will be assigned a participant code and their data will be associated with this code number. The participant code only will be attached to data transferred to MIT using the NIH Secure Email File Transfer (SEFT) approved encryption service. All data with identifying information will be stored on password-protected computers in the CPN Section at NIAAA.

Bed Sensor: Although the device is capable of streaming data wirelessly over Bluetooth to a tablet or mobile phone, for this study, the data shall be recorded internally on a memory card. (Micro-SD). The data will be copied to a laptop at NIAAA and data will be transferred to MIT for data analysis.

Thermal Camera: images will be downloaded from the iPAD to an NIAAA computer will be transferred electronically to MIT for analysis.

H. **Statistical Analysis**

1. Analysis of data/ study outcomes

Outcome data will be examined for homogeneity of variance, and if necessary transformed to meet this criterion. As this is a within-subjects design, repeated measures analysis of covariance (ANCOVA) will be used to analyze the primary outcome, with drug condition as the within-subjects factor. Similarly, for secondary outcome measures, repeated measured ANCOVA will also be used. A probability level of 0.05 or lower will be considered significant.

As for the neuroimaging data, we will use AFNI software to analyze our data. As a first step we will preprocess the data to correct for motion and then realign the functional volumes to the anatomical volumes. Subsequently, we will perform a regression analyses. Voxels are considered active if the total variance accounted for by the best-fit combination of the regressors of interest will exceed a chosen *F* ratio ($P = 0.05$ per voxel, uncorrected for multiple comparisons). We will also perform a subtraction analyses between different BOLD responses. To create the average activation maps, we will perform a random effect approach (activation amplitude and *t*-values will be averaged in each voxel in standardized space across subjects). Group maps will thresholded using 3dClustSim (an analysis module within AFNI). A cluster size of at least 5 voxels will be determined by Monte Carlo simulation.

2. Power analysis

We chose an effect size $dz = 0.4$ which is consistent with effect sizes of cue reactivity observed for other medications like naltrexone, acamprosate and topiramate. Considering the following parameters: $dz = 0.4$, α *err prob* = .05, and power (*1-β err prob*) = .80, a sample size of 41 subjects will be needed. For fMRI, our sample size of $n=27$ is based on the study by Hariri and colleagues (Hariri et al. 2002) , who used the same task in an fMRI study (between subjects design) with a similar sample size subjects.

3. Accrual number

Consistent with the within-subject design, our previous protocol testing the same study medication (14-AA-0042), the inpatient setting and other human laboratory studies performed in our Clinical Program at NIAAA, we anticipate that

approximately 25% of the enrolled participants may not complete the study. Therefore, we anticipate to enroll up to 55 subjects in order to reach at least 41 completers to satisfy the primary aim

I. Human subjects' protection plan

1. Consent documents and process

Procedures conducted before obtaining consent for this protocol
Screening: subjects are prescreened by phone and then screened in-person through the NIAAA Screening protocol 14-AA-0181, which will provide the necessary data to determine eligibility to this protocol.
Clearance for eligibility: inclusion/exclusion criteria will be reviewed by a team of three individuals: research coordinator (or designee); a registered nurse or nurse practitioner; and PI or AI designated by the PI. All three signatures will be required in order to establish participants' eligibility to this protocol.

Consent procedures
Eligible subjects are enrolled in this protocol after the study-specific consent form for this protocol is obtained. Study investigators designated in the "Qualifications of Investigators" section below will obtain informed consents for the studies of this protocol. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing. Participants will be asked to sign and date the informed consent. A copy of the signed and dated consent will be given to each participant. As part of the consent procedure, participants' ability to understand the consent will be evaluated using the 'NIDA/NIAAA Evaluation of Potential Research Participants' Ability to Consent'. Subjects must answer all questions correctly to proceed with consent signing.

2. Qualifications of Investigators

Lorenzo Leggio, M.D., Ph.D., M.Sc., is a Senior Investigator at NIAAA and NIDA and Chief of the Section of Psychoneuroendocrinology and Neuropsychopharmacology (CPN). He has several years of clinical and academic experience in the field of addiction medicine. He has extensive experience in direct patient care, and direction and execution of clinical trials. Before joining NIH, he was Core Faculty at the Brown University Medical School. There, he conducted pharmacotherapy human laboratory studies funded by NIH and Foundations at the Brown University Center for Alcohol and Addiction Studies, where he still holds an adjunct position as Associate Professor. Dr. Leggio is the PI of this study and will be responsible for the conduct, analysis and publication of the results of the study. He will obtain consent for this study.

Mehdi Farokhnia, M.D., is a Staff Scientist in the NIAAA-NIDA joint Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology (CPN). He completed medical school at Tehran University of Medical Sciences and has over 5 years of experience in psychiatric research. During medical school and after that, he has been involved in conducting a variety of research projects while working as a research associate at the Psychiatric Research Center, Roozbeh Psychiatric Hospital. He will obtain consent for this study.

Melanie Schwandt, Ph.D., is a Staff Scientist in the NIAAA Clinical Program. She has more than 10 years of experience in constructing, managing and analyzing large datasets that combine behavioral and physiological outcomes, including pre-clinical research involving alcohol-related phenotypes and clinical research involving data analyses from clinical experimental medicine studies. She will assist the PI in the analysis of the data. She will not obtain consent for this study.

Reza Momenan, Ph.D., is a Staff Scientist and Acting Chief in the Section of Brain Electrophysiology and Imaging, NIAAA. He has extensive experience in human brain imaging including MRI and fMRI and will guide the image pre-processing, processing and image analysis methods. He will provide support in conducting the fMRI sessions and will assist with the analyses of the imaging data. He will obtain consent for this study.

Susan Persky, Ph.D., is an Associate Investigator and Head of the Immersive Virtual Environment Testing Unit in the Social and Behavioral Research Branch (SBRB), National Human Genome Research Institute (NHGRI), NIH. She earned a B.A. in psychology for Northwestern University. She Earned an M.A. and Ph.D. in social psychology from the University of California, Santa Barbara where she studied at the Research Centers for Virtual Environments and Behavior. After conducting postdoctoral research at Columbia University, she joined the SBRB in 2005. Here, she built and led the Immersive Virtual Environment Testing Area, an immersive virtual reality technology-based experimental research lab within the SBRB. She became an associate investigator at NHGRI in 2009, and the head of the Immersive Virtual Environment Test Unit in 2011. She will not obtain consent for this study.

Fatemeh Akhlaghi, Pharm. D., Ph.D., is a tenured full professor in the College of Pharmacy, University of Rhode Island (URI) and adjunct associate professor of Medicine at Brown University Medical School. Her area of specialty is clinical pharmacology in diabetes and transplantation; she also conducts clinical pharmacokinetics research at Rhode Island Hospital, Providence, RI. Dr. Akhlaghi is also a member of the IRB at URI. Dr. Akhlaghi is the Co-PI, together with Dr. Leggio, of the multiple-PI UH2/UH3 grant funded by NCATS. Her laboratory at URI will receive samples that are de-identified from Dr. Leggio's studies at NIAAA during this NCATS project. She will not obtain consent for this study.

Daria Piacentino, M.D., Ph.D., M.Sc., is a Visiting Post-Doctoral Fellow in the NIAAA-NIDA joint Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology (CPN). She received her M.D. and Ph.D. from Sapienza University of Rome and Policlinico Umberto I hospital. She comes to the NIH with extensive clinical and research experience working in the Department of Neurology and Psychiatry with a focus on the diagnosis and treatment of substance abuse and behavioral addiction. She received a M.Sc. degree in Medical Statistics and Statistical Methods for Epidemiology from the University of Milan. She will assist the research team with data collection and analyses, help with experimental procedures and participant visits as per protocol. As a non-FTE staff member, Dr. Piacentino will explain the consent form to participants under the direct supervision of a federal employee (FTE) who will sign the consent form.

Monica Faulkner, Ph.D., is a Post-Doctoral Fellow in the NIAAA-NIDA joint Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology (CPN). She received her PhD in Psychology with a concentration in Behavioral Neuroscience from the University of North Carolina at Chapel Hill in 2018. Her research has focused on investigating the neuromechanisms of addiction using cognitive behavioral testing, neuroimaging, and pharmacology. She will assist the research team with data collection and analyses, help with experimental procedures and participant visits as per protocol As a non-FTE staff member, Dr. Faulkner will explain the consent form to participants under the direct supervision of a federal employee (FTE) who will sign the consent form.

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3. Subject Selection

Adults who fulfill the qualification criteria will be included, regardless of race, ethnicity, sex, or religious affiliation. At this stage of development of this compound (PF-05190457), the manufacturer recommends to exclude women of childbearing potential because reproductive and developmental toxicity studies with PF-05190457 have not been conducted. Therefore, in order to be eligible for this study, female participants must be menopausal or surgically sterile. Definition of menopause and requirements to proof menopausal status are detailed in the inclusion criteria section of the protocol. Proof (medical records, certification from an MD) of surgical sterility will be required. Furthermore, as the effects of this drug on fetal development are unknown, male subjects will be informed verbally and in the consent form that they must be abstinent or use specific precautions, which are detailed in the inclusion criteria section of the protocol, starting with the first dose of study drug and continuing for 28 days after their last dose of study drug.

4. Recruitment

Participants will be recruited through referrals from the NIH Volunteer Office, and from the Patient Recruitment and Public Liaison (PRPL) Office as well as through ResearchMatch.org (facilitated through PRPL). ResearchMatch is a voluntary service that matches people interested in being research participants with researchers conducting a wide range of studies. ResearchMatch is currently used for other protocols in our Clinical Program at NIAAA, as well as for many other NIH protocols. ResearchMatch is a Clinical and Translational Science Awards (CTSA) initiative funded by the National Center for Advancing Translational Sciences (NCATS), part of the National Institutes of Health. Furthermore, participants will also be recruited by word of mouth and through local advertisements. Advertisement language will be used as flyers with tear off tabs, posted on the NIH campus, campuses of universities and colleges in the greater Washington DC area, as well as in billboards of public places and public transportation services in the greater Washington DC area. Advertisements will also be posted in electronic and printed local media, including newsletters, websites and local newspapers in the greater Washington DC area (for example, the 'Express'). Listserv ad will be used on craigslist.org, under the volunteers' section, as well as in NIH and other local email distribution lists, that are moderated, and following approval from the managers of these lists. 'ClinicalTrials.gov' may also represent a source of recruitment. Finally, participants will also be recruited via the NIAAA Screening Protocol 14-AA-0181), as these protocols serve as the screening protocols not only for this study but also for other protocols at the NIAAA Intramural Clinical Program.

5. Children

Children < 18 years of age will not be studied.

6. Vulnerable populations

Vulnerable populations will not be studied.

7. Evaluation of Risks/Discomforts and Benefits ratio

1. Anticipated Benefit

This study does not offer direct benefit to participants. However, the outpatient phase will provide participants with behavioral intervention and counselling, from which they might benefit. Additionally, contemporaneous with their enrollment in the present protocol, subjects are enrolled in 14-AA-0181 which provides standard treatment on the inpatient unit which the subjects may receive if desired.

Classification of risk

Overall risk and discomfort in this study is "more than minimal". The increase over minimal risk, discomfort is minor, and every precaution and protection to minimize any risks will be taken as described in a previous section. This level is justified,

because it will markedly facilitate medication development for AUD, and thus contribute to addressing major unmet medical needs. As such, the risk:benefit ratio of the proposed study is favorable.

2. Justification of sensitive procedures

This study employs the use of placebo to evaluate the effects of the study medication under investigation. This is the required standard set by the FDA for evaluation of drugs.

J. **Protection of Participants' Privacy and Confidentiality**

This protocol is covered by a Certificate of Confidentiality (CoC). Strict subject confidentiality will be maintained throughout the study. Confidentiality and information technology standards are in place at the NIAAA intramural program to protect electronic repositories of patient data as well as other clinical patient related material. It is reasonably expected that these safeguards will protect participants' medical and personal health information, ensuring their privacy.

Information obtained in the course of participating in this protocol will become part of the patient's NIH medical record. Third parties may request access to this information. However, access will not be granted without the explicit, written consent of the subjects. Samples and data will be stored using codes that we assign. Data will be kept in password protected computers. Samples will be kept in locked storage. Only study personnel will have access to the samples and data. Additional biological specimens obtained under this protocol will be stored in locked storage in coded form (protocol identifier plus randomization nr), in -80 °C freezers located in Dr. Akhlaghi's Lab at URI.

K. Study Agents/Interventions

Study Drug

Description of study medication side-effects is based on the previous studies conducted by Pfizer as well as our previous study 14-AA-0042.

The most common side effects noted in studies giving PF-05190457 to humans are: somnolence (sleepiness), headache and fatigue (tiredness) as well as decrease in serum glucose, and increased heart rate.

Less common side-effects are: abdominal discomfort, abdominal distention, abdominal pain, upset stomach, diarrhea, constipation, defecation urgency, gastrointestinal sounds abnormal, flatulence, gastroenteritis, upper respiratory tract infection, nasal congestion, nasopharyngitis, rhinorrhea, oral herpes, elevated transaminases, rhabdomyolysis, arthralgia, pain, pain in extremity, pain in jaw, swelling, hot flush, conjunctival hyperemia, acne cystic, face edema, hemoglobin decreased, dizziness, paraesthesia, hypoaesthesia, lethargy, dermatitis contact, photosensitivity reaction, hematoma, hemorrhage, abnormal eye exam, muscular weakness, musculoskeletal chest pain, depressive mood, euphoria, drowsiness, increased appetite, insomnia, irritability, difficulties to concentrate and sweating.

Most of the side-effects were mild and a few of them were moderate. There were no severe side-effects.

Our team in the CPN lab as well as the CC nursing staff are well familiar with PF-05190457, as we have already conducted and successfully and safely completed another study with this drug (14-AA-0042). Risks of medication will be minimized through careful and detailed screening of participants to minimize recruitment of those who might be at higher risk for adverse events, as well as careful and detailed monitoring of adverse events and participant well-being during the study. As detailed in the research strategy, a screening visit will be performed before enrolling any subject, under the NIAAA Screening Protocol 14-AA-0181. This screening visit will include a comprehensive clinical assessment, including physical exam, testing, psychiatric and medical history, blood and urine tests, and interviews aimed at assessing all

inclusionary/exclusionary criteria listed. For enrolled subjects, adverse medication effects will be systematically evaluated and recorded. *Ad hoc* clinical, laboratory and diagnostic assessments may be performed, should a participant present with specific symptoms, as the clinical situation may dictate. If adverse events are serious or intolerable, participants will be withdrawn from the study and given treatment and or referral as the situation dictates. Concomitant medications will be evaluated carefully before randomizing participants, as detailed in the exclusion criteria section above.

Blood draw and finger stick

The total amount of research blood to be drawn for this protocol will be approximately 434 cc which is within the NIH Clinical Center guidelines. Risks of blood draws and finger sticks include discomfort or possible bruising (hematoma) at the site of needle entry. For blood draws, there is also a small risk of fainting or infection at the site of the needle stick. Risks from blood draws and finger sticks are minimized by experienced medical personnel who will perform these procedures using sterile technique and following universal precautions.

EKG recording

Subjects may experience some discomfort when electrodes will be removed from the skin, which should resolve quickly.

Rating Scales, Questionnaires and other Behavioral Assessments

Participants are asked to complete rating scales and questionnaires (paper-and-pencil or computerized) as part of the study. Some individuals may feel emotional discomfort answering some of the items on rating scales. Such stress will be managed by the study team. If needed, participants have immediate access to licensed health care professionals.

Cue-Reactivity

Alcohol cue-reactivity experiments are performed on a regular basis at NIAAA. Increased craving and distress may appear during the CR procedure. Staff members will monitor the participant via closed circuit monitor/video cameras installed in the 'bar-like' room and the participant will always be able to communicate with staff members. Should clinically significant symptoms appear, participants will have immediate access to licensed health care professionals. Additionally, these risks are minimized by the inpatient design of this protocol. Of note, alcohol cue reactivity experiments are performed only during the cue reactivity procedure, and the study does not include any alcohol administration at any point.

fMRI

Anxiety about enclosed spaces: some individuals experience claustrophobia when placed inside the magnet bore of the scanner. Participants will be warned about this in advance and advised not to participate if they are prone to experiencing anxiety in enclosed places.

Loud noise of scanner: fMRI is a noisy process which could possibly damage a person's hearing. To prevent this we require participants to wear foam earplugs and/or headphones while inside the scanner.

"Virtual Buffet"

Previous studies conducted at the NHGRI Immersive Virtual Environment Test Unit (IVETU) located at the NIH Clinical Center show the safety of this experimental procedure. In the virtual environment encounters previously conducted at NHGRI, there were no incidences of serious cybersickness. Some individuals may experience mild symptoms of motion sickness (also called cybersickness in this context). These symptoms may include mild dizziness, headache, eyestrain, blurred vision, light sensitivity, and/or nausea. Such symptoms are rarely strong and we will discontinue the virtual session should participants report onset of these symptoms. We will continue the virtual session only if the participant desires, all symptoms have subsided, and a clinician approved the continuation of the experiment. Participants will be screened so as

to exclude individuals with conditions or history that would make them particularly susceptible to cybersickness. Furthermore, they will be instructed and reminded to report any symptoms during the encounter.

Motivational Interviewing with Video Feedback
Motivational interviewing video feedback may be tedious, embarrassing or may provoke anxiety or craving. Study clinicians will be with patient at all times and relaxation techniques will be used if patients experience symptoms.

f. Wearable Sensors

Bed Sleep Sensor: The bed motion sensor device is a low-power electromagnetic sensor, which uses radio waves for detection operating at similar frequency as Wi-Fi. The power of the device is 1 milliwatt, which is approximately 1000 times lower than the power emitted by a mobile phone, and does not pose any health risk for the participant.

Thermal Camera: The thermal camera does not pose any physical risks to the study participants. Some study participants may have some psychological concerns about the thermal image. To help address these concerns, we will point out to the participants several important points: The thermal image only measures the temperature at the surface of the skin. It does not see anything under the skin. Participants shall be shown a sample thermal image and can see that many of the facial features are not recognizable. For the iPad tablet that is mounted on the wall, the participants can also be shown their own live thermal image, so they will see what the image looks like and they will feel comfortable using the device.

Risk of compensation for tasks
Although we expect that this will be rare, it is possible that being compensated will cause an inpatient participant with alcohol dependence to desire to purchase alcohol to such an extent that they will leave the hospital against medical advice. In order to decrease the risk of this, AUD inpatients will not be paid in cash on the day of testing, but will receive payment after discharge using the RVS system through the NIH/CC.

Additional Considerations
The PI, Dr. Leggio, is also the holder for the currently active FDA IND # 119,365 (with cross-reference Pfizer IND # 110,476) obtained for the previous protocol that tested this medication (14-AA-0042).

L. Plan for reporting unanticipated problems and adverse events

Adverse events, protocol deviations, unanticipated problems (UP), Unanticipated Adverse Device Effects (UADEs), serious adverse events are defined as described in NIH HRPP Policy 801-Reporting Research Events. The NIH Principal Investigators (PIs)/designee and, as applicable, non-NIH Investigators must report events to the IRB via the Reportable Event Submission Form (REF) in NIH iRIS. All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review in accordance with this policy.

M. Data Safety and Monitoring Plan

The DSMP is consistent with the NIH SOP 17.4. Specifically:

1. *Monitoring mechanism*: this study is monitored by the NIDA/NIAAA Addictions DSMB

2. *Frequency of the monitoring*: Initial DSMB review takes place before the study begins. Subsequent DSMB reviews take place after each 10 patients who have been exposed to study drug, unless otherwise recommended by the DSMB during the interim monitoring reviews
3. *Stop or change rules*: The following rules are followed:
   3a. Criteria for individual subject withdrawal:
   - A Serious Adverse Event (SAE) that is judged by the Investigators as being related to the study drug;
   - At the discretion of the PI and/or MAI and/or other health care provider involved in the study based on adverse event (AE) severity, or self-reported symptoms and/or observed changes in patient's well-being as reported by health care providers including clinically significant changes in vital signs, weight/BMI, blood/urine laboratory values, etc.
   - QTc prolongation on EKG calculated by the Fridericia formula, i.e.: if QTc > 500 ms or if QTc increases compared to baseline by >60ms. This criteria is consistent with those outlined by Pfizer and in 2017 FDA guidance: "*E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs — Questions and Answers (R3) Guidance for Industry*"
   - Non-compliance with protocol procedures or investigator request(s);
   - Patient request
   - 3b. Criteria for stopping the study or suspending enrollment or procedures: The study will be stopped if there are four SAEs determined to be related to the study drug (regardless of how many subjects experienced them), or one unexpected SAE that is determined to be related to the study drug.

4. *Advanced plans for any interim analyses and/or futility analyses*. There are no current plans for interim analyses and/or futility analyses

5. *Information to be monitored*. Progress of the study, including assessment of participant recruitment and accrual and adverse events are reviewed to determine whether there is any change to the risk:benefit ratio of the study. Specific parameters that are monitored include:
   - Adverse Events
   - Weight
   - Vital signs
   - Blood tests: glucose (finger stick), serum electrolytes, AST, ALT, GGT, bilirubin, Creatinine
   - EKG

6. *Communication*. The information to be monitored referenced above is reported to the DSMB at the time of the DSMB, to the Clinical Director (via PTMS) and IRB at the time of continuing review, and to the FDA at the time of annual report. Adverse events are recorded and reported to the Clinical Director, the NIH IRB, the Addictions DSMB, and the FDA in accordance with all NIH requirements for adverse event reporting

N. **Clinical Monitoring Plan**

Quality assurance (QA) will be performed by the Investigators, as well as by a QA monitor, which according to established practice is considered sufficient for small, single-site trials, like the present project. The QA monitor for this study will have considerable expertise in clinical trials and will monitor the study binders on a regular basis and consistent with recruitment pace. Additionally, quality assurance will be monitored independently by the NIMH/NIAAA/NIDA Combined Monitoring Plan, Intramural Research Program Auditing Committee (IRPAC) Coordinated by the Offices of the Clinical Director at NIMH, NIAAA and NIDA. The NIMH/NIAAA/NIDA Combined Monitoring Plan, Intramural Research Program Auditing Committee

(IRPAC) monitors intramural research studies to ensure compliance with GCP, organizational policies and applicable federal, state and local laws and the reliability of study data.

O. **Compensation**

Participants will be compensated for time and research-related inconveniences, to the extent to which they complete them, as follows:

Procedure	**Compensation**
Stage 1	
Virtual Buffet……..	$100.00
Cue-Reactivity…….	$160.00
Completion of all in-patient assessments (for which eligible) in Stage I	
Total Stage I ……	$100.00
	$ 360.00
Stage 2	
Virtual Buffet……..	$100.00
Cue-Reactivity…….	$160.00
Completion of all in-patient assessments (for which eligible) in Stage II	
Total Stage II ……	$100.00
	$ 360.00

OPTIONAL PARTICIPATION – ADDITIONAL REMUNERATION	
Follow-up Visits (motivational interviewing sessions with video feedback and supportive counseling) ($10/each)	$80
Stage 1 fMRI	**$150**
Stage 2 fMRI	**$150**
Incentive for completing all inpatient parts of the study	**$220**
Possible TOTAL COMPENSATION	**$1320**

Compensation will be prorated for parts completed if subjects do not complete the study. If a subject is asked to repeat a procedure, the repeated procedure will be compensated having gained PI (or designee) approval and according to the guidelines established by the NIDA/NIAAA Division of Intramural Research Remuneration of Research Participants Policy. If

needed, subjects will be provided with a taxi paid for by NIH.

P. **Scientific references**

Addolorato G, Capristo E, Leggio L, Ferrulli A, Abenavoli L, Malandrino N, Farnetti S, Domenicali M, D'Angelo C, Vonghia L, Mirijello A, Cardone S, Gasbarrini G. Relationship between ghrelin levels, alcohol craving, and nutritional status in current alcoholic patients. Alcohol Clin Exp Res 30:1933-7, 2006. PMID: 17067359.

Allsop S and Saunders B. Reinforcing robust resolutions: Motivation in relapse prevention with severely dependent problem drinkers. In W.R. Miller & S. Rollnick (Eds), Motivational interviewing: Preparing people to change addictive behavior. New York: Guilford, 1991.

Akamizu T, Iwakura H, Ariyasu H, Hosoda H, Murayama T, Yokode M, Teramukai S, Seno H, Chiba T, Noma S, Nakai Y, Fukunaga M, Nakai Y, Kangawa K, FD Clinical Study Team. Repeated administration of ghrelin to patients with functional dyspepsia: its effects on food intake and appetite. Eur J Endocrinol 158: 491-8, 2008. PMID: 18362296.

Anzenbacher P and Anzenbacherova E. Cytochromes P450 and metabolism of xenobiotics. Cell Mol Life Sci 58:737-747, 2001. PMID: 11437235.

Badaoui A, De Saeger C, Duchemin J, Gihousse D, de Timary P, Stärkel P. Alcohol dependence is associated with reduced plasma and fundic ghrelin levels. Eur J Clin Invest 38: 397-403, 2008. PMID: 18422979.

Beck AT, Baruch E, Balter JM, Steer RA, Warman DM. A new instrument for measuring insight: the Beck Cognitive Insight Scale. Schizophrenia Research 68: 319-329, 2004.

Blanchard KA, Morgenstern J, Morgan T.J, Lang M, Bux D A. Motivational subtypes and continuous measures of readiness for change: Concurrent and predictive validity. Psychology of Addictive Behaviors 17: 56–65, 2003.

Bohn MJ, Krahn DD, Staehler BA. Development and Initial Validation of a Measure of Drinking Urges in Abstinent Alcoholics. Alcohol Clin Exp Res 19: 600-6, 1995.

Bottlender M, Soyka M. Impact of craving on alcohol relapse during, and 12 months following, outpatient treatment. Alcohol Alcohol 39:357-61, 2004. PMID: 15208171.

Bollinger J, Acker J, and MacKillop J. Initial Validation of the Brief Alcohol Behavior Social Density Assessment (BASDA) in a college student sample. Poster presented at the 35th Annual Convention of the Behavioral Sciences. Athens, GA. 2012, April.

Bouhlal S, McBride CM, Ward DS, Persky S. Drivers of overweight mothers' food choice behaviors depend on child gender. Appetite 84: 154-60, 2015.

Bray MA and Kehle T J. Long-term follow-up of self-modeling as an intervention for stuttering. School Psychology Review 30: 135–141, 2001.

Broadbent E, Petrie K J, Main J, Weinman J. The brief illness perception questionnaire. J Psychosom Res 60(6): 631-637, 2006. doi:10.1016/j.jpsychores.2005.10.020

Brown KW, Ryan RM. The benefits of being present: mindfulness and its role in psychological well-being. Journal of personality and social psychology. 2003 Apr;84(4):822.

Burnette JL. Implicit theories of body weight: entity beliefs can weigh you down. Pers Soc Psychol Bull 30(2): 410-422, 2010. doi:10.1177/0146167209359768

Calissendorff J, Danielsson O, Brismar K, Röjdmark S. Inhibitory effect of alcohol on ghrelin secretion in normal man. Eur J Endocrinol 152: 743-7, 2005. PMID: 15879360.

Calissendorff J, Danielsson O, Brismar K, Röjdmark S. Alcohol ingestion does not affect serum levels of peptide YY but decreases both total and octanoylated ghrelin levels in healthy subjects. Metabolism 55: 1625-9, 2006. PMID: 17142135.

Calissendorff J, Gustafsson T, Holst JJ, Brismar K, Röjdmark S. Alcohol intake and its effect on some appetite-regulating hormones in man: influence of gastroprotection with sucralfate. Endocr Res 37:154-62, 2012. PMID: 22621425.

Carpino PA, Lefker BA, Toler SM, Pan LC, Hadcock JR, Murray MC, Cook ER, DiBrino JN, DeNinno SL, Chidsey-Frink KL, HadaWA, Inthavongsay J, Lewis SK, Mangano FM, Mullins MA, Nickerson DF, Ng O, Pirie CM, Ragan JA, Rose CR, Tess DA, Wright AS, Yu L, Zawistoski MP, Pettersen JC, DaSilva-Jardine PA, Wilson TC, Thompson DD. Discovery and biological characterization of capromorelin analogues with extended half-lives. Bioorg Med Chem Lett 12: 3279–3282, 2002. PMID: 12392732.

Carver CS and White TL. Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS Scales. Journal of Personality and Social Psychology 67(2): 319-333, 1994. doi:10.1037/0022-3514.67.2.319

Chollet C, Meyer K, Beck-Sickinger AG. Ghrelin--a novel generation of anti-obesity drug: design, pharmacomodulation and biological activity of ghrelin analogues. J Pept Sci 15:711-30, 2009. PMID: 19787814.

Cowley MA, Smith RG, Diano S, Tschop M, Pronchuk N, Grove KL, Strasburger CJ, Bidlingmaier M, Esterman M, Heiman ML, Garcia-Segura LM, Nillni EA, Mendez P, Low MJ, Sotonyi P, Friedman JM, Liu H, Pinto S, Colmers WF, Cone RD, Horvath TL. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron 37:649–61, 2003. PMID: 12597862.

Cox LS, Tiffany S, Christen AG. Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. Nicotine Tob Res 3(1): 7-16, 2001. doi:10.1080/14622200020032051

Cruz MT, Herman MA, Cote DM, Ryabinin AE, Roberto M. Ghrelin Increases GABAergic Transmission and Interacts with Ethanol Actions in the Rat Central Nucleus of the Amygdala. Neuropsychopharmacology, 2013. PMID:22968812.

de Timary P, Cani PD, Duchemin J, Neyrinck AM, Gihousse D, Laterre PF, Badaoui A, Leclercq S, Delzenne NM, Stärkel P. The loss of metabolic control on alcohol drinking in heavy drinking alcohol-dependent subjects. PLoS One 7:e38682, 2012. PMID: 22808013; PMCID: PMC3392266.DiClemente CC. Motivation for change: Implications for substance abuse treatment. Psychological Science 10 (3): 209–213, 1999.

DiClemente CC and Hughes SO. Stages of change profiles in alcoholism treatment. Journal of Substance Abuse 2: 217-235, 1990.

Dowrick PW. Self model theory: learning from the future. WIREs Cognitive Science 3: 215-230, 2012.

Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, Ghatei MA, Small C, Bloom SR. Ghrelin increases food intake in obese as well as lean subjects. Int J Obes (Lond) 29:1130-6, 2005. PMID: 15917842.

Dunn JP, Kessler RM, Feurer ID, Volkow ND, Patterson BW, Ansari MS, Li R, Marks-Shulman P, Abumrad NN. Relationship of dopamine type 2 receptor binding potential with fasting neuroendocrine hormones and insulin sensitivity in human obesity. Diabetes Care 35:1105-11, 2012. PMID: 22432117.

Edwards S, Kenna GA, Swift RM, Leggio L. Current and promising pharmacotherapies, and novel research target areas in the treatment of alcohol dependence: a review. Curr Pharm Des 17:1323-32, 2011.

Egecioglu E, Prieto-Garcia L, Studer E, Westberg L, Jerlhag E. The role of ghrelin signaling for sexual behaviour in male mice. Addict Biol: 2015. in press. doi: 10.1111/adb.12202; PubMed PMID: 25475101.

Ely AV, Childress AR, Jagannathan K, Lowe MR. The way to her heart? Response to romantic cues is dependent on hunger state and dieting history: An fMRI pilot study. Appetite95:126-31, 2015.

Fleming SM, Weil RS, Nagy Z, Dolan RJ, Rees G. Relating introspective accuracy to individual differences in brain structure. Science 2010 Sep 17;329(5998):1541-3.

Furuse M, Tachibana T, Ohgushi A, Ando R, Yoshimatsu T, Denbow DM. Intracerebroventricular injection of ghrelin and growth hormone releasing factor inhibits food intake in neonatal chicks. Neurosci Lett 301:123–6, 2001. PMID: 11248438.

Gahete MD, Córdoba-Chacón J, Kineman RD, Luque RM, Castaño JP. Role of ghrelin system in neuroprotection and cognitive functions: implications in Alzheimer's disease. Peptides 32:2225-8, 2011. PMID: 21983104.

Garbutt JC, West SL, Carey TS, Lohr KN, Crews FT. Pharmacological treatment of alcohol dependence: A Review of the Evidence, JAMA 281:1318-25, 1999. PMID: 10208148.Ghareeb M, Leggio L, El-Kattan A, Akhlaghi F. Development and validation of an UPLC-MS/MS assay for quantitative analysis of the ghrelin receptor inverse agonist PF-5190457 in human or rat plasma and rat brain. Anal Bioanal Chem 407:5603-13, 2015. PMID: 25943263

Goldstein RZ, Craig AD, Bechara A, Garavan H, Childress AR, Paulus MP, et al. The neurocircuitry of impaired insight in drug addiction. Trends Cogn Sci 2009 Sep;13(9):372-80

Guay F, Vallerand R, Blanchard C. On the Assessment of Situational Intrinsic and Extrinsic Motivation: The Situational Motivation Scale (SIMS). Motivation and Emotio*n* 24(3): 175-213, 2000.

Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, Smith RG, Van der Ploeg LH, Howard AD. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. Brain Res Mol Brain Res 48: 23-9, 1997. PMID: 9379845.

Haas MJ. Pharma compounds at the crossroads. Science-business exchange 5(26): published online June 28, 2012.doi:10.1038/scibx.2012.669

Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, Egan MF, Weinberger DR. Serotonin transporter genetic variation and the response of the human amygdala. Science. 2002 Jul 19;297(5580):400-3.

He P, Court MH, Greenblatt DJ, von Moltke LL. Factors influencing midazolam hydroxylation activity in human liver microsomes. Drug Metab Dispos 34:1198-1207, 2006. PMID: 16638818.

Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: A revision of the Fagerstrom Tolerance Questionnaire. British Journal of Addictions 86:1119-27, 1991. PMID: 1932883.

Hedeker D and Mermelstein RJ. Methods for analyzing longitudinal data on relapse: Application of random-effects regression models in relapse research. Addiction 91(Suppl): S211–S229, 1996.

Heilig M, Egli M. Pharmacological treatment of alcohol dependence: target symptoms and target mechanisms. Pharmacol Ther 111:855-76, 2006. PMID: 16545872.

Hillemacher T, Kraus T, Rauh J, Weiss J, Schanze A, Frieling H, Wilhelm J, Heberlein A, Gröschl M, Sperling W, Kornhuber J, Bleich S. Role of appetite-regulating peptides in alcohol craving: an analysis in respect to subtypes and different consumption patterns in alcoholism. Alcohol Clin Exp Res 31:950-4, 2007. PMID: 17433008.

Holst B, Schwartz TW. Constitutive ghrelin receptor activity as a signaling set-point in appetite regulation. Trends Pharmacol Sci 25:113–117, 2004. PMID: 15058279.

Holst B, Schwartz TW. Ghrelin receptor mutations – too little height and too much hunger. J Clin Invest 116:637–641, 2006. PMID: 16511600.

Janssen I, Katzmarzyk PT, Ross R. Waist circumference and not body mass index explains obesity-related health risk. Am J Clin Nutr. 2004 Mar;79(3):379-84. PubMed PMID: 14985210.

Jerlhag E. Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. Addict Biol 13: 358-63, 2008. PMID: 18358991.

Jerlhag E, Egecioglu E, Dickson SL, Andersson M, Svensson L, Engel JA. Ghrelin Stimulates Locomotor Activity and Accumbal Dopamine-Overflow via Central Cholinergic Systems in Mice: Implications for its Involvement in Brain Reward. Addict Biol 11: 45-54, 2006a. PMID: 16759336.

Jerlhag E, Egecioglu E, Dickson SL, Douhan A, Svensson L, Engel JA. Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. Addict Biol 12: 6-16, 2007. PMID: 17407492.

Jerlhag E, Egecioglu E, Dickson SL, Svensson L, Engel JA. Alpha-conotoxin MII-sensitive nicotinic acetylcholine receptors are involved in mediating the ghrelin-induced locomotor stimulation and dopamine overflow in nucleus accumbens. Eur Neuropsychopharmacol 18: 508-18, 2008. PMID: 18343642.

Jerlhag E, Egecioglu E, Landgren S, Salomé N, Heilig M, Moechars D, Datta R, Perrissoud D, Dickson SL, Engel JA. Requirement of central ghrelin signaling for alcohol reward. Proc Natl Acad Sci USA 106:11318-23, 2009. PMID: 19564604.

Jerlhag E, Grøtli M, Luthman K, Svensson L, Engel JA. Role of the subunit composition of central nicotinic acetylcholine receptors for the stimulatory and dopamine-enhancing effects of ethanol. Alcohol 41: 486-93, 2006b. PMID: 16799162.

Jerlhag E, Janson AC, Waters S, Engel JA. Concomitant release of ventral tegmental acetylcholine and accumbal dopamine by ghrelin in rats. PLoS One 7: e49557, 2012. PMID: 23166710; PMC3498203.

Jerlhag E, Landgren S, Egecioglu E, Dickson SL, Engel JA. The alcohol-induced locomotor stimulation and accumbal dopamine release is suppressed in ghrelin knockout mice. Alcohol 45:341-7, 2011. PMID: 21145690.

Jiang H, Betancourt L, Smith RG. Ghrelin Amplifies Dopamine Signaling by Cross Talk Involving Formation of Growth Hormone Secretagogue Receptor/Dopamine Receptor Subtype 1 Heterodimers. Molecular Endocrinology 20:1772–1785, 2006.

Kashdan TB, Gallagher MW, Silvia PJ, Winterstein BP, Breen WE, Terhar D, Steger MF. The curiosity and exploration inventory-II: Development, factor structure, and psychometrics. Journal of research in personality. 2009 Dec 31;43(6):987-98.

Katayama M, Nogami H, Nishiyama J, Kawase T, Kawamura K. Developmentally and regionally regulated expression of growth hormone secretagogue receptor mRNA in rat brain and pituitary gland. Neuroendocrinology 72:333–40, 2000. PMID: 11146416.

Kawahara Y, Kawahara H, Kaneko F, Yamada M, Nishi Y, Tanaka E, Nishi A. Peripherally administered ghrelin induces bimodal effects on the mesolimbic dopamine system depending on food-consumptive states. Neuroscience 161:855-64, 2009. PMID: 19362120.

Kim DJ, Yoon SJ, Choi B, Kim TS, Woo YS, Kim W, Myrick H, Peterson BS, Choi YB, Kim YK, Jeong J. Increased fasting plasma ghrelin levels during alcohol abstinence. Alcohol Alcohol 40: 76-9, 2005. PMID: 15520048.

Kim J, Kim S, Lee W, Cheon Y, Lee S, Ju A, K M, Kim D. The Effects of Alcohol Abstinence on BDNF, Ghrelin, and Leptin Secretions in Alcohol-Dependent Patients with Glucose Intolerance. Alcohol Clin Exp Res, 2012. PMID: 22974102.

Kim JS, Kim GJ, Lee JM, Lee CS, Oh JK. HAIS (Hanil Alcohol Insight Scale): Validation of an Insight-Evaluation Instrument for Practical use in Alcoholism. Journal of Studies on Alcohol 59(1): 52-55, 1998.

Kim KM, Kim JS, Kim GJ, Kim SS, Jung JG, Kim SM, Pack HJ, Lee DH. The Readiness to Change and Insight in Alcohol Dependent Patients. Journal of Korean Medical Sciences 22: 453-458, 2007.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402: 656-60, 1999. PMID: 10604470.

Koob GF. Neural mechanisms of drug reinforcement. Ann N Y Acad Sci 654:171-91, 1992. PMID: 1632582.

Koopmann A, von der Goltz C, Grosshans M, Dinter C, Vitale M, Wiedemann K, Kiefer F. The association of the appetitive peptide acetylated ghrelin with alcohol craving in early abstinent alcohol dependent individuals. Psychoneuroendocrinology 37:980-6, 2012. PMID: 22172639.

Kraus T, Schanze A, Gröschl M, Bayerlein K, Hillemacher T, Reulbach U, Kornhuber J, Bleich S. Ghrelin levels are increased in alcoholism. Alcohol Clin Exp Res 29: 2154-7, 2005. PMID: 16385185.

Kumar S, Earla R, Jin M, Mitra AK, and Kumar A. Effect of ethanol on spectral binding, inhibition, and activity of CYP3A4 with an antiretroviral drug nelfinavir. Biochem Biophys Res Commun 402:163-167, 2010. PMID: 20937259.

Kumar S and Kumar A. Differential effects of ethanol on spectral binding and inhibition of cytochrome P450 3A4 with eight protease inhibitors antiretroviral drugs. Alcohol Clin Exp Res 35:2121-2127, 2011. PMID: 21682753.

Landgren S, Simms JA, Hyytiä P, Engel JA, Bartlett SE, Jerlhag E. Ghrelin receptor (GHS-R1A) antagonism suppresses both operant alcohol self-administration and high alcohol consumption in rats. Addict Biol 17:86-94, 2012. PMID: 21309944.

Larsson A, Edström L, Svensson L, Söderpalm B, Engel JA. Voluntary ethanol intake increases extracellular acetylcholine levels in the ventral tegmental area in the rat. Alcohol Alcohol 40, 349-58, 2005. PMID: 16043436.

Larsson A, Jerlhag E, Svensson L, Söderpalm B, Engel JA. Is an alpha-conotoxin MII sensitive mechanism involved in the neurochemical, stimulatory and rewarding effects of ethanol. Alcohol 34, 239-46, 2004. PMID: 15902919.

Leggio L. Role of the ghrelin system in alcoholism: Acting on the growth hormone secretagogue receptor to treat alcohol-related diseases. Drug News Perspect 23:157-66, 2010. PMID: 20440417.

Leggio L, Addolorato G, Cippitelli A, Jerlhag E, Kampov-Polevoy AB, Swift RM. Role of feeding-related pathways in alcohol dependence: A focus on sweet preference, NPY, and ghrelin. Alcohol Clin Exp Res 35:194-202, 2011. PMID: 21058960.

Leggio L, Ferrulli A, Cardone S, Nesci A, Miceli A, Malandrino N, Capristo E, Canestrelli B, Monteleone P, Kenna GA, Swift RM, Addolorato G. Ghrelin system in alcohol-dependent subjects: role of plasma ghrelin levels in alcohol drinking and craving. Addict Biol 17:452-64, 2012. PMID: 21392177.

Leggio L, Zywiak WH, Fricchione SR, Edwards SM, de la Monte SM, Swift RM, Kenna GA. Intravenous ghrelin administration increases alcohol craving in alcohol-dependent heavy drinkers: a preliminary investigation. Biol Psychiatry 76: 734-41, 2014. PMID: 24775991

Leggio L, Zywiak WH, McGeary JE, Edwards S, Fricchione S, Shoaff JR, Addolorato G, Swift RM, Kenna GA. A Human Laboratory Pilot Study with Baclofen in Alcoholic Individuals. Pharmacol Biochem Behav 103: 784-91, 2013. PMID: 23262301

Link BG, Phelan JC, Bresnahan M, Stueve A, Pescosolido BA. Public conceptions of mental illness: labels, causes, dangerousness, and social distance. Am J Public Health 89(9): 1328-1333, 1999.Littleton J, Barron S, Prendergast M, Nixon SJ. Smoking kills (alcoholics)! shouldn't we do something about it? Alcohol Alcohol 42:167-73, 2007. PMID: 17526626.

Loose MD, Ronnekleiv OK, Kelly MJ. Neurons in the rat arcuate nucleus are hyperpolarized by GABAB and mu-opioid receptor agonists: evidence for convergence at a ligand-gated potassium conductance. Neuroendocrinology 54: 537-44, 1991. PMID: 1664497.

Lytton SD, Helander A, Zhang-Gouillon ZQ, Stokkeland K, Bordone R, Arico S, Albano E, French SW, and Ingelman-Sundberg M. Autoantibodies against cytochromes P-4502E1 and P-4503A in alcoholics. Mol Pharmacol 55:223-233, 1999. PMID: 9927612.

Marlatt GA. Craving for alcohol, loss of control, and relapse: A cognitive-behavioral analysis. In P.E. Nathan, G.A. Marlatt & T. Loberg (Eds), Alcoholism: New directions in behavioral research and treatment, p.271-314, 1978. New York, NY: Plenum Press.

Metcalfe J, Greene MJ. Metacognition of agency. J Exp Psychol Gen 2007 May;136(2):184-99

Miller WR, Walters ST, Bennett ME. How effective is alcoholism treatment in the United States? J Stud Alcohol 62: 211-20, 2001. PMID: 11327187.

Miller WR, Tonigan SJ. Assessing Drinkers' Motivation for Change: The Stages of Change Readiness and Treatment Eagerness Scale (SOCRATES). Psychology of Addictive Behaviors 10(2): 81-89, 1996.

Miller WR. Enhancing motivation for change in substance abuse treatment. Treatment Improvement Protocol (TIP) Series, No. 35. Rockville, MD: Center for Substance Abuse Treatment, 1999.

Monti PM, Rohsenow DJ, Hutchison KE. Toward bridging the gap between biological, psychobiological and psychosocial models of alcohol craving. Addiction 95:S229-36, 2000. PMID: 11002917.

Monti PM, Rohsenow DJ, Rubonis AV, Niaura RS, Sirota AD, Colby SM, Abrams DB. Alcohol cue reactivity: effects of detoxification and extended exposure. J Stud Alcohol 54:235-45, 1993. PMID: 8384678.

Moss-Morris R, Weinman J, Petrie KJ, Horne R, Cameron LD, Buick D. The Revised Illness Perception Questionnaire (IPQ-R). Psychology and Health 17(1): 1-16, 2002.Muench F, Tryon W, Travaglini L, Morgenstern J. Maintaining motivation using audio review. Journal of Substance Use 11(3): 191-204, 2006.

Naleid AM, Grace MK, Cummings DE, Levine AS. Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. Peptides 26:2274–9, 2005. PMID: 16137788.

National Institute on Alcohol Abuse and Alcoholism. Tenth Special Report to the U.S. Congress on Alcohol and Health, Washington, DC: U.S. Department of Health and Human Services, 2000.

NCATS website, Library of Industry-Provided Agents, Table of Compounds and Biologics; available at: http://www.ncats.nih.gov/research/reengineering/rescue-repurpose/therapeutic-uses/directory.html [last access August 7, 2012].

Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, Frost GS, Ghatei MA, Coombes RC, Bloom SR. Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. J Clin Endocrinol Metab 89: 2832-6, 2004. PMID: 15181065.

Niemela O, Parkkila S, Juvonen RO, Viitala K, Gelboin HV, and Pasanen M. Cytochromes P450 2A6, 2E1, and 3A and production of protein-aldehyde adducts in the liver of patients with alcoholic and non-alcoholic liver diseases. J Hepatol 33:893-901, 2000. PMID: 11131450.

Orleans CT. Promoting the maintenance of health behavior change: recommendations for the next generation of research and practice. Health Psychology 19(1 Suppl): 76-83, 2000.

Pantel J, Legendre M, Cabrol S, Hilal L, Hajaji Y, Morisset S, Nivot S, Vie-Luton MP, Grouselle D, de Kerdanet M, Kadiri A, Epelbaum J, Le Bouc Y, Amselem S. Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. J Clin Invest 116:760–768, 2006. PMID: 16511605.

Parkinson A, Mudra DR, Johnson C, Dwyer A, and Carroll KM. The effects of gender, age, ethnicity, and liver cirrhosis on cytochrome P450 enzyme activity in human liver microsomes and inducibility in cultured human hepatocytes. Toxicol Appl Pharmacol 199:193-209, 2004. PMID: 15364537.

Persky S, McBride CM, Faith MS, Wagner LK, Ward DS. Mothers' guilt responses to children's obesity risk feedback. J Health *Psychol* 20(5): 649-658, 2015.

Polcin DL, Galloway GP, Palmer J, Mains W. The case for High-Dose Motivational Enhancement Therapy. Substance Use and Misuse 39: 331-343, 2004.

Prieto-Garcia L, Egecioglu E, Studer E, Westberg L, Jerlhag E. Ghrelin and GHS-R1A signaling within the ventral and laterodorsal tegmental area regulate sexual behavior in sexually naïve male mice. Psychoneuroendocrinology 62:392-402, 2015.

Quarta D, Di Francesco C, Melotto S, Mangiarini L, Heidbreder C, Hedou G. Systemic administration of ghrelin increases extracellular dopamine in the shell but not the core subdivision of the nucleus accumbens. Neurochem Int 54:89-94, 2009.

Rahmioglu N, Heaton J, Clement G, Gill R, Surdulescu G, Zlobecka K, Hodgkiss D, Ma Y, Hider RC, Smith NW, and Ahmadi KR. Genetic epidemiology of induced CYP3A4 activity. Pharmacogenet Genomics 21:642-651, 2011. PMID: 21750469.

Rohsenow DJ, Monti PM, Hutchison KE, Swift RM, Colby SM, Kaplan GB. Naltrexone's effects on reactivity to alcohol cues among alcoholic men. J Abnorm Psychol 109:738-42, 2000. PMID: 11195999.

Rohsenow DJ, Monti PM, Rubonis AV, Gulliver SB, Colby SM, Binkoff JA, Abrams DB. Cue exposure with coping skills training and communication skills training for alcohol dependence: 6- and 12-month outcomes. Addiction 96:1161-74, 2001. PMID: 11487422.

Rubak S, Sandbaek A, Lauritzen T, Christensen B. Motivational interviewing: a systematic review and meta-analysis. British Journal of General Practice 55(513): 305-312, 2005.

Salmela KS, Kessova IG, Tsyrlov IB, and Lieber CS. Respective roles of human cytochrome P-4502E1, 1A2, and 3A4 in the hepatic microsomal ethanol oxidizing system. Alcohol Clin Exp Res 22:2125-2132, 1998. PMID: 9884161.

Samet JH, Horton NJ, Traphagen ET, Lyon SM, and Freedberg KA. Alcohol consumption and HIV disease progression: are they related? Alcohol Clin Exp Res 27:862-867, 2003. PMID: 12766632.

Sanchis-Segura C, Spanagel R. Behavioural assessment of drug reinforcement and addictive features in rodents: An overview. Addict Biol 11:2–38, 2006. PMID: 16759333.

Schechter DS, Myers MM, Brunelli SA, Coates SW, Zeanah CH, Davies M, Grienenberger JF, Marshall RD, McCaw JE, Trabka KA, Liebowitz MR.. Traumatized Mothers Can Change Their Minds About Their Toddlers: Understanding How A Novel use of Videofeedback Supports Positive Change of Maternal Attributions. Infant Mental Health Journal 27(5): 429-447, 2006.

Schinkel AH. P-Glycoprotein, a gatekeeper in the blood-brain barrier. Adv Drug Deliv Rev 36:179-194, 1999. PMID: 10837715.

Schneider ER, Rada P, Darby RD, Leibowitz SF, Hoebel BG. Orexigenic peptides and alcohol intake: differential effects of orexin, galanin, and ghrelin. Alcohol Clin Exp Res 31, 1858-65, 2007. PMID: 17850217.

Schomerus, G., Corrigan, P. W., Klauer, T., Kuwert, P., Freyberger, H. J., & Lucht, M. (2011). Self-stigma in alcohol dependence: consequences for drinking-refusal self-efficacy. *Drug Alcohol Depend,* 114(1), 12-17. doi: 10.1016/j.drugalcdep.2010.08.013

Solomon A, De Fanti BA, Martinez JA. Peripheral ghrelin participates in glucostatic feeding mechanisms and in the anorexigenic signalling mediated by CART and CRF neurons. Nutr Neurosci 8:287–95, 2005. PMID: 16669599.

Stengel A, Taché Y. RLegegulation of food intake: the gastric X/A-like endocrine cell in the spotlight. Curr Gastroenterol Rep 11:448-54, 2009. PMID: 19903420.

Swart EL, Zuideveld KP, de Jongh J, Danhof M, Thijs LG, and Strack van Schijndel RM. Comparative population pharmacokinetics of lorazepam and midazolam during long-term continuous infusion in critically ill patients. Br J Clin Pharmacol 57:135-145, 2004.

Swift RM. Medication and alcohol craving. Alcohol Res Health 23: 207-13, 1999. PMID: 10890816.

Swift RM, Leggio L. Adjunctive Pharmacotherapy in the Treatment of Alcohol and Drug Dependence. In Evidence-Based Addiction Treatment, Peter Miller Ed., Part III, Elsevier. Chapter 17, pp. 287-310, 2009.

Tomasi D, Volkow ND. Striatocortical pathway dysfunction in addiction and obesity: differences and similarities. Crit Rev Biochem Mol Biol, 2012. PMID: 23173916.

Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, Guan JL, Wang QP, Funahashi H, Sakurai T, Shioda S, Matsukura S, Kangawa K, Nakazato M. Ghrelin-induced food intake is mediated via the orexin pathway. Endocrinology 144:1506–12, 2003. PMID: 12639935.

Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 407:908–13, 2000. PMID: 11057670.

Tupala E, Tiihonen J. Dopamine and alcoholism: neurobiological basis of ethanol abuse. Prog Neuropsychopharmacol Biol Psychiatry 28: 1221-47, 2004. PMID: 15588749.

Unniappan S, Lin X, Cervini L, Rivier J, Kaiya H, Kangawa K, Peter RE. Goldfish ghrelin: molecular characterization of the complementary deoxyribonucleic acid, partial gene structure and evidence for its stimulatory role in food intake. Endocrinology 143:4143–6, 2002. PMID: 12239128.

Vaillant GE. The natural history of alcoholism. Cambridge, MA: Harvard University Press, 1983.

Volkow ND, Wang GJ, Tomasi D, Baler RD. The addictive dimensionality of obesity. Biol Psychiatry 73: 811-8, 2013. PMID: 23374642.

Weathermon R and Crabb DW. Alcohol and medication interactions. Alcohol Res Health 23:40-54, 1999.

Wickizer T, Maynard C, Atherly A, Frederick M, Koepsell T, Krupski A, Stark K . Completion rates of clients discharged from drug and alcohol treatment programs in Washington State. American Journal of PublicHealth 84: 215–221, 1994.

Wolff N, von Hippel C, Brener L, von Hippel W. Implicit identification with drug and alcohol use predicts retention in residential rehabilitation programs. Psychol Addict Behav 29(1): 136-141, 2015. doi:10.1037/adb0000004

Wurst FM, Graf I, Ehrenthal HD, Klein S, Backhaus J, Blank S, Graf M, Pridzun L, Wiesbeck GA, Junghanns K. Gender differences for ghrelin levels in alcohol-dependent patients and differences between alcoholics and healthy controls. Alcohol Clin Exp Res 31:2006-11, 2007. PMID: 17949465.

Zigman JM, Jones JE, Lee CE, Saper CB, Elmquist JK. Expression of ghrelin receptor mRNA in the rat and the mouse brain. J Comp Neurol 494:528–48, 2006. PMID: 16320257.

Zimmermann US, Buchmann A, Steffin B, Dieterle C, Uhr M. Alcohol administration acutely inhibits ghrelin secretion in an experiment involving psychosocial stress. Addict Biol 12: 17-21, 2007. PMID: 17407493.

Q. **Appendices**

Appendix 1 - Inclusion/exclusion criteria checklist:

Inclusion criteria

YES NO

___ ___ Male or female individuals 18-70 years old (inclusive)

___ ___ Current Alcohol Use Disorder by DSM-5 criteria

___ ___ Most recent urine drug test for illegal drugs of abuse is negative

___ ___ Most recent Clinical Institute Withdrawal Assessment for Alcohol-revised (CIWA-Ar) score ≤ 8

___ ___ Heart rate ≤ 100 on two separate measurements, both assessed after CIWA-Ar score is ≤ 8

___ ___ Female subjects must be of non-childbearing potential as defined by at least one of the

Following criteria;

a) Females 45 – 70 years old, who are menopausal, defined as follow:

i) Females who are between 45 – 55 years old: they will be considered menopausal if they satisfy <u>all</u> the following three requirements during screening: 1) they are in amenorrhea, defined as absence of menstruation for the previous 12 months; 2) they have a negative urine pregnancy test; and 3) they have a serum FSH level within the laboratory's reference range for postmenopausal females.

ii) Females who are between 56 – 70 years old: they will be considered menopausal if they are in amenorrhea, defined as absence of menstruation for the previous 12 months before screening.

<u>OR</u>

b) Females 21-70 years old, who have a documented hysterectomy and/or bilateral oophorectomy.

All other female subjects (including females with tubal ligations and females that do NOT have a documented hysterectomy) will be considered to be of childbearing potential.

___ ___ Male subjects must use one of the following methods of contraception:

a. Abstinence.

b. A condom AND one of the following:

- Vasectomy for more than 6 months.
- Female partner who meets one of the following conditions:
    1. Has had a tubal ligation, hysterectomy, or bilateral oophorectomy;
    2. Is post-menopausal;
    3. Uses one of the following forms of contraception:

- Copper or hormonal containing IUD;
- Spermicidal foam/gel/film/cream/suppository;
- Diaphragm with spermicide;
- Oral contraceptive;
- Injectable progesterone;
- Subdermal implant.

Exclusion criteria

YES NO

___ ___ Lifetime clinical diagnosis of schizophrenia or bipolar disorder

___ ___ EKG with QTc > 450 msec as determined by the Fridericia formula.

___ ___ BMI <18.5 kg/m^2 or anorexia

___ ___ BMI ≥ 40 kg/m^2

___ ___ History of epilepsy and/or seizures
NOTE: individuals who have a history of alcohol withdrawal seizures may be in the study as long as they have been abstinent from alcohol for at least 2 weeks prior to consent and during that period of abstinence, there were no seizure episodes (otherwise, participant remains not eligible).

___ ___ Most recent blood tests show creatinine ≥ 2 mg/dL, AST or ALT > 3x the upper normal limit, hemoglobin <10.5 g/dl

___ ___ Subjects who have diabetes and/or are treated with any drug with glucose lowering properties such as sulfonylurea, insulin, metformin, thiazolidinediones (TZD), Dipeptidyl peptidase-4 (DPP4) inhibitors, or Glucagon-like peptide-1(GLP-1)agonists (due to the glucose-lowering properties of PF-05190457 observed in healthy volunteers)

___ ___ Exclusionary Medications:

A.
Naltrexone, acamprosate, alcohol dehydrogenase inhibitors, topiramate, gabapentin, ondansetron, benzodiazepines, , and alpha-1 blockers, baclofen, , drugs that are known to prolong the QTc interval and barbiturates as well as hormone replacement therapy; medications and dietary/herbal supplements (like St. John's wort) that interact with Cytochrome P450 3A4. Patients who take these medications may be enrolled in the study only if the potentially interacting medication has been stopped for a period of at least 5 half-lives of the interacting medication before PF-05190457 administration.

B.

___ ___ Unable to pass a finger rub hearing test

___ ___ Vision is unable to be corrected to (Snellen) 20/100

___ ___ Clinically-significant history of motion or car sickness, or history of vestibular disorders

___ ___ Any other reason or clinical condition for which the PI or the MAI will consider unsafe for a possible participant to participate in this study

fMRI Exclusion Criteria

___ ___ Have contraindications for brain fMRI, as determined by the NIAAA MRI Safety screening form

___ ___ Colorblindness (this would prevent subject from completing the Stroop task) using the Ishihara Test for Color Deficiency, Concise Edition, 2014.

FINAL DETERMINATION

□ **NOT ELIGIBILE**

□ **ELIGIBLE BUT WITHOUT fMRI**

□ **ELIGIBLE INCLUDING fMRI**

Eligibility checklist verification:

Signature	**Role**	**Date**
1. _____	_____	_____
2. _____	_____	_____
3. _____	_____	_____

Appendix 2 – Table Outline for the Inpatient Phase

	Stage 1								Washout		Stage 2
	D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8+**	**D9**	**D10+**	**D11-17+ (D1-8)**
Inpatient 1SE	X →										↓
Task Training	X	X				X					
Vitals, weight, BrAC	X	X	X	X	X	X	X #	X			
Blood PK/PD	X	X	X	X	X**	X**	X** #	X			
Blood Clinical	X						#				
Blood Research	X	X	X	X	X**	X**	X** #	X			
AUQ	X				X**	X**	X** #				
GFCQ-T	X										
GFCQ-S	X				X**	X**	X** #				
POMS	X				X**	X**	X** #				
SHAPS	X							X			
Subject Characterizations (done once before study drug administration D1 only)											
BASDA	X										
BAS/BIS	X										
FTND	X										
Sweet Preference	X										
IAT	X										
IVETU Questionnaires▲	X										↓
Cigs per day	X	X	X	X	X	X	X	X			
Smoking Topog.			X		X		X				
Study Drug BID	X	X	X	X	X	X	X	X			
Adverse Events	X	X	X	X	X	X	X	X			
Stanford SS			X		X		X				
EKG	X						#				
FS glucose			X		X		X				
Waist circumference	X						#				
***IVETU**					X^						
***fMRI**						X					
***Cue Reactivity**							X				
Study drug check							#				

All times and scheduling is approximate and may be modified to allow for scheduling and time constraints.
*order of these tasks is flexible to allow for scheduling with the exception of the Cue Reactivity which will be done last.
** Research bloods and PK/PD bloods done twice on these days.
#these assessments will be performed the day after Cue Reactivity

Blood PK/PD and Blood Research: Genetics, feeding, stress, hormones of interest, inflammatory markers, GH, Insulin

Blood Clinical: serum electrolytes, AST, ALT, GGT, bilirubin, Creatinine

▲IVETU Questionnaires:

Perceived Family History: weight and alcohol problems
Implicit Theories of Weight, Alcohol Use and Eating
Causal Beliefs Scale for Alcohol Dependence and Casual Beliefs Scale for Body Weight
Perceptions of Personal Control and Treatment Control
Self-Stigma and Alcohol Dependence
Perceptions of passing down propensity for disease

^Including Post-IVETU Questionnaires:

Thoughts about the Buffet Scenario
Environmental Presence
Post-IVETU Symptom Checklist
Real-world Food Behavior

NIDA/NIAAA Evaluation of Potential Research Participants' Ability to Consent

Subject Randomization number_____ **Protocol #: 16-AA-0080**

For each question, circle the score that most closely reflects your evaluation.

You may explain or rephrase questions to help the potential participant understand them.

Individuals who do not pass the first time may be re-evaluated once

1	What is the purpose of the study? [must understand that goal of the study and must understand that the study drug is not approved to treat alcoholism]	0 = no or insufficient understanding 1 = sufficient understanding
2	What will you be asked to do if you enroll? [must understand primary procedures, e.g., alcohol cues, MRI and IVETU]	0 = no or insufficient understanding 1 = sufficient understanding
3	What are the most significant risks to you of being in the study? [must recognize the most significant risks, e.g., drowsiness, increased heart rate, lowered glucose levels and risks related to MRI and IVETU]	0 = no or insufficient understanding 1 = sufficient understanding
4	What happens if you decide you do not want to be in the study? [must understand that can decline without penalty]	0 = no or insufficient understanding 1 = sufficient understanding
5	What are the chances that being in the study will benefit you personally? [must understand potential benefits, including the possibility that will not benefit personally]	0 = no or insufficient understanding 1 = sufficient understanding
6	If you enroll, can you later change your mind and stop participating? [must understand that can stop participating at any time]	0 = no or insufficient understanding 1 = sufficient understanding
7	What would you do if you experience pain or distress while in the study? [must be willing to tell team]	0 = no or insufficient understanding 1 = sufficient understanding
8	What things could you do (for your condition) besides being in the study? [must recognize available alternatives, including, for example, approved medications for alcoholism]	0 = no or insufficient understanding 1 = sufficient understanding

I hereby certify that the potential participant has the ability to consent and scored 1 on all questions.

_____ (Evaluator Signature) _____ / _____ /_____ (Date)

Appendix 4 – "Virtual Buffet" at the NHGRI Immersive Virtual Environment Test Unit (IVETU)

a) Examples of screenshots of "virtual buffet":

b) Post-experiment questionnaires:

1. Thoughts about the Buffet Scenario

The next questions relate to your thoughts and feedback regarding the virtual buffet scenario.
Please choose the most appropriate number to indicate how you feel:

	Not at all						Completely
1. How much did you like the virtual buffet overall?	1	2	3	4	5	6	7
2. How realistic did you feel the buffet scenario was?	1	2	3	4	5	6	7
3. How difficult was it to use the pointing device to choose foods from the buffet?	1	2	3	4	5	6	7
4. Was the food that ended up on your plate the amount you intended to select?	1	2	3	4	5	6	7
5. Was the food on the buffet the same kind of food you typically eat?	1	2	3	4	5	6	7
6. How likely would you be to eat the meal you chose in the virtual buffet if it were real food?							
7. How representative is the meal you chose, in terms of the types of food on the plate, in comparison to what you would normally choose at a buffet restaurant?							
8. If you were given a meal of real food and drink that was exactly the same as what you chose in the virtual buffet (same foods and drink), how likely would you be to eat that meal?							
9. Did you feel that there were enough foods on the buffet that you like to eat?							
10. How satisfied do you think you would be by the meal that you prepared in the virtual buffet?							

These next questions relate to your thoughts about how realistic the portion sizes were. Please think about the portions that you were able to end up with on your plate:

	Portions too small		Portions just right		Portions too big
11. How realistic were the food portions that you were able to choose?	1	2	3	4	5
12. How realistic were the drink sizes that you were able to choose?	1	2	3	4	5
	None		**Some of it**		**All of it**
13. How much of the meal do you think you would have eaten?	1	2	3	4	5
14. Did you run out of room on your plate when selecting food?	***Yes***			***No***	

2. Environmental Presence

The next questions relate to your experience in the virtual world. Please read each question. Choose the answer that best reflects your feelings:

	Not at all	Slightly	Moderately	Very much	Extremely
1. To what extent were you involved in the virtual world?	1	2	3	4	5
2. To what extent did you feel like you were inside the virtual world?	1	2	3	4	5
3. To what extent did you feel surrounded by the virtual world?	1	2	3	4	5
4. To what extent did it feel like you visited another place?	1	2	3	4	5
5. How much did the virtual world seem like the real world?	1	2	3	4	5

3. Post-IVETU Symptoms Checklist (Nichols et al., 1997; Bouhlal et al., 2015)

To what degree did you experience each of the following symptoms when you were in the virtual environment? Please use the following scale to indicate your experience.

	None	Slight	Moderate	Severe
1. Headache	0	1	2	3
2. Blurred vision	0	1	2	3
3. Dizzy (eyes open)	0	1	2	3
4. Dizzy (eyes closed)	0	1	2	3
5. Nausea	0	1	2	3

4. Real-World Food Behavior

The following questions relate to your opinions and perceptions of the food you eat on a regular basis.

	Very below average			Average			Very above average
1. Do you consider the amount of food you eat each day to be:	1	2	3	4	5	6	7
2. Do you consider the number of calories you eat each day to be:	1	2	3	4	5	6	7

Below is a list of the different foods and drinks you encountered in the virtual world. Please indicate how likely it is that you would select each of the following foods in real life, given that you could also choose any other sort of food you would normally eat or would like to eat during a lunch meal.

	Extremely unlikely 1	Unlikely 2	Somewhat unlikely 3	Neither Likely nor Unlikely 4	Somewhat likely 5	Likely 6	Extremely likely 7
Brownies							
Steamed Carrots							
Cheese Pizza bites							
Grilled Chicken							
Corn							
French fries							

Grapes							
Strawberry Yogurt							
Green Beans							
Macaroni and Cheese							
Applesauce							
Chicken Nuggets							
Orange Slices							
Mini cheeseburger sliders							
White rice							
Cookies							

1. What aspects of the virtual buffet experience did you like the most?

_____
_____
_____

2. What aspects of the virtual buffet experience did you like the least?

_____
_____
_____

Appendix 5: Motivational Interviewing Questionnaires

Personal Drinking Questionnaire
(SOCRATES 8A)

INSTRUCTIONS: Please read the following statements carefully. Each one describes a way that you might (or might not) feel *about your drinking*. For each statement, circle one number from 1 to 5, to indicate how much you agree or disagree with it *right now*. Please circle one and only one number for every statement.

	NO! Strongly Disagree	No Disagree	? Undecided or Unsure	Yes Agree	YES! Strongly Agree
1. I really want to make changes in my drinking.	1	2	3	4	5
2. Sometimes I wonder if I am an alcoholic.	1	2	3	4	5
3. If I don't change my drinking soon, my problems are going to get worse.	1	2	3	4	5
4. I have already started making some changes in my drinking.	1	2	3	4	5
5. I was drinking too much at one time, but I've managed to change my drinking.	1	2	3	4	5
6. Sometimes I wonder if my drinking is hurting other people.	1	2	3	4	5
7. I am a problem drinker.	1	2	3	4	5
8. I'm not just thinking about changing my drinking, I'm already doing something about it.	1	2	3	4	5
9. I have already changed my drinking, and I am looking for ways to keep from slipping back to my old pattern.	1	2	3	4	5
10. I have serious problems with drinking.	1	2	3	4	5

	NO! Strongly Disagree	No Disagree	? Undecided or Unsure	Yes Agree	YES! Strongly Agree
11. Sometimes I wonder if I am in control of my drinking.	1	2	3	4	5
12. My drinking is causing a lot of harm.	1	2	3	4	5
13. I am actively doing things now to cut down or stop drinking.	1	2	3	4	5
14. I want help to keep from going back to the drinking problems that I had before.	1	2	3	4	5
15. I know that I have a drinking problem.	1	2	3	4	5
16. There are times when I wonder if I drink too much.	1	2	3	4	5
17. I am an alcoholic.	1	2	3	4	5
18. I am working hard to change my drinking.	1	2	3	4	5
19. I have made some changes in my drinking, and I want some help to keep from going back to the way I used to drink.	1	2	3	4	5

Situational Motivation Scale (SIMS)

Why are you currently engaged in this activity?

1. Because I think that this activity is interesting 1 2 3 4 5 6 7

2. Because I am doing it for my own good 1 2 3 4 5 6 7

3. Because I am supposed to do it 1 2 3 4 5 6 7

4. There may be good reasons to do this activity, but personally

I don't see any 1 2 3 4 5 6 7

5. Because I think that this activity is pleasant 1 2 3 4 5 6 7

6. Because I think that this activity is good for me 1 2 3 4 5 6 7

7. Because it is something that I have to do 1 2 3 4 5 6 7

8. I do this activity but I am not sure if it is worth it 1 2 3 4 5 6 7

9. Because this activity is fun 1 2 3 4 5 6 7

10. By personal decision 1 2 3 4 5 6 7

11. Because I don't have any choice 1 2 3 4 5 6 7

11. Because I don't have any choice 1 2 3 4 5 6 7

12. I don't know; I don't see what this activity brings me 1 2 3 4 5 6 7

13. Because I feel good when doing this activity 1 2 3 4 5 6 7

14. Because I believe that this activity is important for me 1 2 3 4 5 6 7

15. Because I feel that I have to do it 1 2 3 4 5 6 7

16. I do this activity, but I am not sure it is a good thing to pursue it 1 2 3 4 5 6 7

Beck Cognitive Insight Scale (modified)

	Do not agree at all	Agree slightly	Agree a lot	Agree completely
(1) At times, I have misunderstood other people's attitudes towards me.				
(2) My interpretations of my experiences are definitely right.				
(3) Other people can understand the cause of my drinking better than I can.				
(4) I have jumped to conclusions too fast.				
5) Some of my experiences that have seemed very real may have been due to my imagination.				
(6) Some of the ideas I was certain were true turned out to be false.				
(7) If something feels right, it means that it is right.				
(8) Even though I feel strongly that I am right, I could be wrong.				
(9) I know better than anyone else what my problems are.				
(10) When people disagree with me, they are generally				

wrong.				
(11) I cannot trust other people's opinion about my drinking				
(12) If somebody points out that my beliefs are wrong, I am willing to consider it.				
(13) I can trust my own judgment at all times.				
(14) There is often more than one possible explanation for why people act the way they do.				
(15) My drinking may be due to my being extremely upset or stressed.				

Hanil Alcohol Insight Scale (HAIS)

1. I find many problems in my drinking.
2. I can control drinking any time if I want to.
3. All my problems can be solved only when I quit drinking.
4. My drinking did no harm to any member of the family.
5. I have been hospitalized because of too much drinking.
6. I feel upset when people view me as a problem drinker.
7. I am an alcoholic!
8. I can't do without drinking.
9. I am really sorry for the suffering I have caused others by my drinking.
10. I hate the person who put me in a hospital.
11. I find no problems in my drinking.
12. I am unable to stop drinking once I start.
13. I just need some moderation rather than being kept from drinking.
14. Many people around me suffered from my drinking.
15. Drinking itself shouldn't justify my hospitalization.
16. Drinking has deprived me of important things.
17. It's nonsense for them to call me an alcoholic.
18. Sober living is the only way to save my life from ruin.
19. I hate all the people and surroundings that have made me fall into drinking.
20. I was fortunate to have a chance to be hospitalized.

Appendix 6 – Advertisement Material

Print Ad for Newspapers:

DO YOU DRINK A LOT OF ALCHOL?

We are conducting a study testing an experimental drug that might reduce craving for alcohol

We are looking for volunteers who drink a lot of alcohol and are:

- Between the ages of 18 and 70
- Without significant medical or drug problems
- Willing to stay at the NIH Clinical Center
- May want help for your drinking problem

For more details,
Email [insert email here]
Or call *XXX-XXX-XXXX*

The Ad above and its text can also be used for: 1) a tear-sheet flyer, where tear-sheets include NIH protocol #, email and phone number; 2) NIH Listserv and other Listserv (e.g. Craigslist); and 3) advertisement newsletters and websites

AD for NIAAA and other NIH Recruiting Website

Join a Research Study: Alcohol Use Disorder

An experimental drug that might reduce craving for alcohol
The main purpose of this research study is to test whether a drug reduces craving for alcohol.

Research participation includes two - 14 day inpatient visits. You will be asked to take the study drug or placebo by mouth twice a day; taste several sweet solutions; have physical exams and vital signs taken; be exposed to alcohol, water, and food cues in a room that looks like a bar; answer questions on a computer; participate in virtual reality tasks; and have brain scans completed.

After the inpatient stays, optional outpatient follow up visits in our clinic in Bethesda, MD will be offered. These will include counseling to help abstain from alcohol use as well as follow up physical exams.

Who may participate: right-handed adults ages 18-70 who have alcohol use disorder and are otherwise physically healthy.

You may not be eligible if you are pregnant, have metal in your body, have had serious head injuries, or have serious physical or neurological diseases.

The study is conducted at the NIH Clinical Center in Bethesda, MD. Compensation is provided for participation.

To find out if you qualify email [insert email here] or call xxx-xxx-xxxx

Protocol Number: 16-AA-0080
Principal Investigator: Lorenzo Leggio, MD, PhD, MSc

Ads for Social Media

- **Clinical Center Facebook (links to study-specific webpage on the OPR recruitment website,** https://go.usa.gov/xQjhb

Researchers want to test a drug to learn if it helps reduce cravings for alcohol. Are you a right-handed adult age 18-70 who have alcohol use disorder and are otherwise physically healthy?
Learn more at: clinicaltrials.gov and search by study 16-AA-0080. Contact us at: [insert email here]

- **Clinical Center Twitter**
  Volunteers wanted for Research Study on Alcohol Use Disorder looking at reducing craving for alcohol. Learn more at https://go.usa.gov/xQjhbLearn more at clinicaltrials.gov and search by study 16-AA-0080 or learn more at https://go.usa.gov/xQjhb

- **CC News/NIH Record**

NIAAA is seeking right-handed adults ages 18-70 who have alcohol use disorder and are otherwise healthy.

Compensation provided. More information: Office of Patient Recruitment 1-866-444-2214, (Email: [insert email here]. Read about the study at clinicaltrials.gov (search by study 16-AA-0080) or learn more at https://go.usa.gov/xQjhb

- **OPR LISTSERV ("Volunteers" List of those interested in receiving updated protocol information via email. Links to this study on OPR recruitment website)**

  "A research study to learn more about testing a drug to learn if it helps reduce craving for alcohol".
  A research team at the National Institutes of Health (NIH) in Bethesda, Maryland is looking for, right-handed adults ages 18-70 who have alcohol use disorder and are otherwise healthy to participate in a study.
  Study highlights:

- An experimental drug that might reduce craving for alcohol
- The main purpose of this research study is to test whether a drug reduces craving for alcohol.
- Research participation includes two - 14 day inpatient visits. You will be asked to take the study drug or placebo by mouth twice a day; taste several sweet solutions; have physical exams and vital signs taken; be exposed to alcohol, water, and food cues in a room that looks like a bar; answer questions on a computer; participate in virtual reality tasks; and have brain scans completed.
- After the inpatient stays, optional outpatient follow up visits in our clinic in Bethesda, MD will be offered. These will include counseling to help abstain from alcohol use as well as follow up physical exams.
- Who may participate: right-handed adults ages 18-70 who have alcohol use disorder and are otherwise physically healthy.
- You may not be eligible if you are pregnant, have metal in your body, have had serious head injuries, or have serious physical or neurological diseases.
- The study is conducted at the NIH Clinical Center in Bethesda, MD. Compensation is provided for participation.
- The NIH Clinical Center, America's research hospital, is located in Bethesda, Maryland, on the Metro red line (Medical Center stop).

  For more information, call:
  Office of Patient Recruitment
1-866-444-1132
Email: [insert email here]
Online: clinicaltrials.gov and search by study 16-AA-0080 or Learn more at https://go.usa.gov/xQjhb

Recruitment Ads
Facebook, Twitter, OPR and NIH Listservs,
Craigslist, ResearchMatch, CC News/NIH Record
DRAFT DATE: 4/23/2018
IRB APPROVED:

Social Media (Pictures will be used interchangeably and found at the end of this document)

Hashtags**:** #AlcoholAwarenessMonth (April) #alcohol

Organization/Association tags: @NIHClinicalCntr @samhsagov @NIAAAnews

Facebook Posts: (Each time posted to Facebook, hashtag a different condition listed in hashtags and tag one of the listed associations**)**

AUD non-treatment seeking

Are you a heavy alcohol drinker? Consider participating in a research study at the NIH Clinical Center. NIAAA researchers are investigating an experimental medication that might reduce alcohol craving. There is an inpatient stay for approximately 3-4 weeks. Compensation is provided. For more information, call the NIH Clinical Center Office of Patient Recruitment at 1-800-411-1222. Refer to study 16-AA-0080. https://go.usa.gov/xQjhb

Researchers at the NIH Clinical Center seek individuals who drink alcohol for a research study. This study is investigating an experimental medication that might reduce alcohol craving. There is an inpatient stay for approximately 3-4 weeks. Compensation is provided. For more information, call the NIH Clinical Center Office of Patient Recruitment at 1-800-411-1222. Refer to study 16-AA-0080. https://go.usa.gov/xQjhb

AUD treatment seeking

Are you seeking treatment for alcohol use? Consider participating in a research study at the NIH Clinical Center. NIAAA researchers are investigating an experimental medication that might reduce alcohol craving. There is an inpatient stay for approximately 3-4 weeks. Compensation is provided. For more information, call the NIH Clinical Center Office of Patient Recruitment at 1-800-411-1222. Refer to study 16-AA-0080. https://go.usa.gov/xQjhb

Are you worried about your alcohol use? Consider participating in a research study at the NIH Clinical Center. NIAAA researchers are investigating an experimental medication that might reduce alcohol craving. There is an inpatient stay for approximately 4 weeks.. Compensation is provided. For more information, call the NIH Clinical Center Office of Patient Recruitment at 1-800-411-1222. Refer to study 16-AA-0080. https://go.usa.gov/xQjhb

Twitter Posts:

AUD non-treatment seeking

Do you drink alcohol heavily @NIHClinicalCntr seek participants for an inpatient research study testing a medication to reduce alcohol craving. Compensation provided. Call the NIH Office of Patient Recruitment at 1-800-411-1222. Study #16-AA-0080. https://go.usa.gov/xQjhb

AUD treatment seeking

Are you Seeking treatment for alcohol use? @NIHClinicalCntr seek participants for an inpatient research study testing a medication to reduce alcohol craving. Compensation provided. Office of Patient Recruitment at 1-800-411-1222. Study #16-AA-0080. https://go.usa.gov/xQjhb

OPR and NIH Listservs (Email list of those interested in receiving study recruitment updates. (Links to specific study page on CC Search the Studies, clinicaltrials.gov or OPR recruitment website)

AUD non-treatment seeking

Are you a heavy alcohol drinking individual? Join a research study…

Researchers at the National Institutes of Health (NIH) are looking for individuals to participate in a research study investigating an experimental medication that might reduce alcohol craving.

Ghrelin is a hormone found naturally in the body that stimulates appetite. It may also stimulate alcohol craving and use. We want to learn more about alcohol craving and test if a drug that blocks ghrelin lowers the craving for alcohol.

Compensation is provided for participation.

You may be eligible if you:

- You are 18-70-year-old
- Drink alcohol heavily

Participating includes:

- Inpatient stay for approximately 3-4 weeks. Optional outpatient follow up visits
- Taking the study medication or placebo (liquid solution with no active ingredients) by mouth twice a day
- Physical exams, blood tests, brain imaging scans, questionnaires, virtual reality tasks, and exposure to alcohol, water, and food cues in a bar simulation

The study takes place at the NIH Clinical Center, America's Research Hospital in Bethesda, MD. We are on the Metro red line (Medical Center stop).

For more information:
NIH Clinical Center Office of Patient Recruitment
1- 866-444-1132 (refer to NIH study 16-AA-0080)
TTY for the deaf and hard of hearing: 1-866-411-1010
https://go.usa.gov/xQjhb

AUD treatment seeking

Title Option 1. Are you worried about your drinking? Consider joining a research study…
Title option 2. Is your drinking affecting your life? Consider joining a research study…

Researchers at the National Institutes of Health (NIH) are looking for individuals to participate in a research study investigating an experimental medication that might reduce alcohol craving.

Ghrelin is a hormone found naturally in the body that stimulates appetite. It may also stimulate alcohol craving and use. We want to learn more about alcohol craving.and test if a drug that blocks ghrelin lowers the craving for alcohol.

There is no cost to participate and compensation is provided.

You may be eligible if:

- You are 18-70-year-old
- Are a heavy drinker and may want help for your drinking

Participating includes:

- Inpatient stay for approximately 3-4 weeks. Optional outpatient follow up visits
- Taking the study medication or placebo (liquid solution with no active ingredients) by mouth twice a day
- Physical exams, blood tests, brain imaging scans, questionnaires, virtual reality tasks, and be exposed to alcohol, water, and food cues in a bar simulation

The study takes place at the NIH Clinical Center, America's Research Hospital in Bethesda, MD. We are on the Metro red line (Medical Center stop).

For more information:
NIH Clinical Center Office of Patient Recruitment
1- 866-444-1132 (refer to NIH study 16-AA-0080)
TTY for the deaf and hard of hearing: 1-866-411-1010
https://go.usa.gov/xQjhb

Craigslist

AUD non-treatment seeking

Heading:

Are you a heavy drinker? Join a research study…

Researchers at the National Institutes of Health (NIH) are looking for individuals to participate in a research study investigating an experimental medication that might reduce alcohol craving.

Ghrelin is a hormone found naturally in the body that stimulates appetite. It may also stimulate alcohol craving and use. We want to learn more about alcohol cravingand test if a drug that blocks ghrelin lowers the craving for alcohol.

Compensation is provided for participation.

You may be eligible if:

- You are a healthy 18-70-year-old
- Are a heavy drinker

Participating includes:

- Inpatient stay for approximately 3-4 weeks. Optional outpatient follow up visits
- Taking the study medication or placebo (liquid solution with no active ingredients) by mouth twice a day

- Physical exams, blood tests, brain imaging scans, questionnaires, virtual reality tasks, and be exposed to alcohol, water, and food cues in a bar simulation

The study takes place at the NIH Clinical Center, America's Research Hospital in Bethesda, MD. We are on the Metro red line (Medical Center stop).

For more information:
NIH Clinical Center Office of Patient Recruitment
1-877-888-4447 (refer to NIH study 16-AA-0080)
TTY for the deaf and hard of hearing: 1-866-411-1010
https://go.usa.gov/xQjhb

AUD treatment seeking

Heading: Are you worried about your drinking? Join a research study…
Are you looking for alcohol treatment?
Is your drinking affecting your life?

Are you worried about your drinking? Consider joining a research study…

Researchers at the National Institutes of Health (NIH) are looking for individuals to participate in a research study investigating an experimental medication that might reduce alcohol craving.

Ghrelin is a hormone found naturally in the body that stimulates appetite. It may also stimulate alcohol craving and use. We want to learn more about alcohol cravingand test if a drug that blocks ghrelin lowers the craving for alcohol.

There is no cost to participate and compensation is provided.

You may be eligible if:
- You are a healthy 18-70-year-old
- Are a heavy drinker and may want help for your drinking

Participating includes:
- Inpatient stay for approximately 3-4 weeks. Optional outpatient follow up visits
- Taking the study medication or placebo (liquid solution with no active ingredients) by mouth twice a day
- Physical exams, blood tests, brain imaging scans, questionnaires, virtual reality tasks, and be exposed to alcohol, water, and food cues in a bar simulation

The study takes place at the NIH Clinical Center, America's Research Hospital in Bethesda, MD. We are on the Metro red line (Medical Center stop).

NIH Clinical Center Office of Patient Recruitment
1-877-888-4447 (refer to NIH study 16-AA-0080)
TTY for the deaf and hard of hearing: 1-866-411-1010
https://go.usa.gov/xQjhb

ResearchMatch (Web links, phone numbers, and contact information are not allowed)

AUD non-treatment seeking

Are you a heavy drinker? Join a research study…

Researchers at the National Institutes of Health (NIH) are looking for individuals to participate in a research study investigating an experimental medication that might reduce alcohol craving.

Ghrelin is a hormone found naturally in the body that stimulates appetite. It may also stimulate alcohol craving and use. We want to learn more about alcohol cravingand test if a drug that blocks ghrelin lowers the craving for alcohol.

Compensation is provided for participation.

You may be eligible if:

- You are a healthy 18-70-year-old
- Are a heavy drinker

Participating includes:

- Inpatient stay for approximately 3-4 weeks. Optional outpatient follow up visits
- Taking the study medication or placebo (liquid solution with no active ingredients) by mouth twice a day
- Physical exams, blood tests, brain imaging scans, questionnaires, virtual reality tasks, and be exposed to alcohol, water, and food cues in a bar simulation

The study takes place at the NIH Clinical Center, America's Research Hospital in Bethesda, MD. We are on the Metro red line (Medical Center stop).

AUD treatment seeking

Are you worried about your drinking? Consider joining a research study…

Researchers at the National Institutes of Health (NIH) are looking for individuals to participate in a research study investigating an experimental medication that might reduce alcohol craving.

Ghrelin is a hormone found naturally in the body that stimulates appetite. It may also stimulate alcohol craving and use. We want to learn more about alcohol cravingand test if a drug that blocks ghrelin lowers the craving for alcohol.

There is no cost to participate and compensation is provided.

You may be eligible if:

- You are a healthy 18-70-year-old
- Are a heavy drinker and may want help for your drinking

Participating includes:

- Inpatient stay for approximately 3-4 weeks. Optional outpatient follow up visits
- Taking the study medication or placebo (liquid solution with no active ingredients) by mouth twice a day
- Physical exams, blood tests, brain imaging scans, questionnaires, virtual reality tasks, and be exposed to alcohol, water, and food cues in a bar simulation

The study takes place at the NIH Clinical Center, America's Research Hospital in Bethesda, MD. We are on the Metro red line (Medical Center stop).

NIH Newsletters (CC News, NIH Record):

Are you a heavy drinker? NIAAA researchers are seeking research volunteers for a study testing a medication that may reduce cravings for alcohol. There is an Inpatient stay for approximately 3-4 weeks. Compensation is provided. For more information, call the NIH Clinical Center Office of Patient Recruitment 1-866-444-2214 (TTY for the deaf or hard of hearing: 1-866-411-1010). https://go.usa.gov/xQjhb. Refer to study 16-AA-0080.

Pictures to be used interchangeably for social media, listserv postings and flyers:

;

Consider joining a research study...

Researchers at the **National Institutes of Health (NIH)** are looking for individuals to participate in a research study investigating an experimental medication that might reduce alcohol craving.

Ghrelin is a hormone found naturally in the body that stimulates appetite. It may also stimulate alcohol craving and use. We want to learn more about alcohol cravingand test if a drug that blocks ghrelin lowers the craving for alcohol.

There is no cost to participate and compensation is provided.

You may be eligible if you are:

- A 18-70 years old
- A heavy drinker with no contraindication to be in the study

Participating includes:

- Approximately 3-4 week inpatient stay
- Optional outpatient follow up visits
- Taking the study medication or placebo (a liquid solution with no active ingredients) by mouth twice a day
- Physical exams, blood tests, brain imaging scans, questionnaires, virtual reality tasks, and be exposed to alcohol, water, and food cues in a bar simulation

The study takes place at the NIH Clinical Center, America's Research Hospital in Bethesda, MD. We are on the Metro red line (Medical Center stop).

FOR MORE INFORMATION, CALL:

NIH Clinical Center Office of Patient Recruitment

1-800-411-1222 (refer to 16-AA-0080)

TTY for the deaf and hard of hearing: 1-866-411-1010

ONLINE: HTTPS://GO.USA.GOV/XQJHB

Appendix 7: Characterization Measures

BIS/BAS

Each item of this questionnaire is a statement that a person may either agree with or disagree with. For each item, indicate how much you agree or disagree with what the item says. Please respond to all the items; do not leave any blank. Choose only one response to each statement. Please be as accurate and honest as you can be. Respond to each item as if it were the only item. That is, don't worry about being "consistent" in your responses. Choose from the following four response options

Very true for me	Somewhat true for me	Somewhat false for me	Very false for me
(1)	(2)	(3)	(4)

_____1. A person's family is the most important thing in life.

_____2. Even if something bad is about to happen to me, I rarely experience fear or nervousness.

_____3. I go out of my way to get things I want.

_____4. When I'm doing well at something I love to keep at it.

_____5. I'm always willing to try something new if I think it will be fun.

_____6. How I dress is important to me.

_____7. When I get something I want, I feel excited and energized.

_____8. Criticism or scolding hurts me quite a bit.

_____9. When I want something I usually go all-out to get it.

_____10. I will often do things for no other reason than that they might be fun.

_____11. It's hard for me to find the time to do things such as get a haircut.

_____12. If I see a chance to get something I want I move on it right away.

_____13. I feel pretty worried or upset when I think or know somebody is angry at me.

_____14. When I see an opportunity for something I like I get excited right away.

_____15. I often act on the spur of the moment.

_____16. If I think something unpleasant is going to happen I usually get pretty "worked up."

_____17. I often wonder why people act the way they do.

_____18. When good things happen to me, it affects me strongly.

_____19. I feel worried when I think I have done poorly at something important.

_____20. I crave excitement and new sensations.

_____21. When I go after something I use a "no holds barred" approach.

_____22. I have very few fears compared to my friends.

_____23. It would excite me to win a contest.

_____24. I worry about making mistakes.

BASDA – Brief Addictive Behavior Social Density Assessment

Please provide estimates of these behaviors for the people who are closest to you. In each case, check the appropriate boxes or write in your answer. Please answer each question even if you are not sure. Your answer should be your best guess.

Person #1:

Your closest non-biologically related individual, including spouses and significant others.

Over the last year, how often does this person have a drink containing alcohol? **☐ Never ☐ Monthly or Less ☐ 2-4 Times/Month ☐ 2-3 Times/Week ☐ 4 or More Times/Week**
Over the last year, how many drinks containing alcohol does this person have on a typical day when he or she is drinking? **☐ 1 to 2 ☐ 3 to 4 ☐ 5 to 6 ☐ 7 to 9 ☐ 10 or more**
Over the last year, how often does this person have six or more drinks on one occasion? **☐ Never ☐ Monthly or Less ☐ 2-4 Times/Month ☐ 2-3 Times/Week ☐ Daily or Almost Daily**
Over the last year, how many cigarettes/day does this person typically smoke each day? _____ **[If the person smokes weekly, write in the number and "per week"]**
Is this person male or female? ☐ Male ☐ Female
Is this person a spouse/significant other or friend? ☐ Spouse/Significant Other ☐ Friend
How confident are you that your estimates about this person are accurate? **☐ Not Confident ☐ Confident ☐ Highly Confident**

The above questions are repeated for the following individuals:

Person #2:

Your SECOND closest non-biologically related individual, including spouses and significant others.

Person #3:

Your THIRD closest non-biologically related individual, including spouses and significant others.

Person #4:

Your FOURTH closest non-biologically related individual.

<u>Person #5</u>: Your MOTHER.

<u>Person #6:</u> Your FATHER.

<u>Person #7:</u> Your SIBLING closest in age.

<u>Person #7:</u> Your next SIBLING closest in age.

How many additional siblings do you have? _____

Perceived Family History

1. Do alcohol problems run in your family?

Yes	No	I don't know

2. Do weight problems run in your family?

Yes	No	I don't know

3. Right now, do you consider yourself to be currently:

Underweight	About right	Overweight	Very overweight

Implicit Theories of Weight, Alcohol Use and Eating [Source**:** Burnette, 2010]

Choose the number that indicates how much you agree with each statement:

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree

1. You have a certain body weight, and you can't really do much to change it
1. Your body weight is something about you that you can't change very much
2. No matter who you are, you can significantly change your body weight
3. To be honest, you can't really change your body weight
4. You can always substantially change your body weight
5. You can change your basic body weight considerably

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree

1. You have a certain amount of alcohol that you drink, and you can't really do much to change it
2. The amount of alcohol you drink is something about you that you can't change very much
3. No matter who you are, you can significantly change the amount of alcohol you drink
4. To be honest, you can't really change the amount of alcohol you drink
5. You can always substantially change the amount of alcohol you drink
6. You can change the amount of alcohol you drink considerably

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree

1. You have a certain amount of food you eat, and you can't really do much to change it
2. The amount of food you eat is something about you that you can't change very much
3. No matter who you are, you can significantly change the amount of food you eat
4. To be honest, you can't really change the amount of food you eat
5. You can always substantially change the amount of food you eat
6. You can change the amount of food you eat considerably

Causal Beliefs Scale for Alcohol Dependence [From Link, et al., 1999 and the Revised Illness Perception Questionnaire, Moss-Morris et al., 2002]

There are several reasons that people give when asked about what causes someone to have a problem with alcohol. In your opinion, how much do you agree or disagree that your alcohol problem might be caused by each of the following:

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree

1. A chemical imbalance
2. A person's own character
3. The way a person was raised
4. Personality
5. Stressful circumstances in a person's life
6. A genetic or inherited problem
7. A lack of willpower
8. God's will
9. Chance or bad luck
10. A person's own behavior
11. A person's emotional state

Causal Beliefs Scale for Body Weight

There are several reasons that people give when asked about what causes someone to have a certain body weight. In your opinion, how much do you agree or disagree that your body weight might be caused by each of the following:

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree

1. A chemical imbalance
2. A person's own character
3. The way a person was raised
4. Personality
5. Stressful circumstances in a person's life
6. A genetic or inherited problem
7. A lack of willpower
8. God's will
9. Chance or bad luck
10. A person's own behavior
11. A person's emotional state

Perceptions of Personal control [Source: Illness Perception Questionnaire, Brief, Broadbent et al. 2006]

1	2	3	4	5	6	7	8	9	10
Absolutely no control									An extreme amount of control

1. How much control do you feel you have over your alcohol use?
2. How much control do you feel you have over your weight?
3. How much control do you feel you have over your eating behavior?

Perceptions of Treatment control [source: IPQ Brief, Broadbent et al. 2006]

1	2	3	4	5	6	7	8	9	10
Not at all helpful									Extremely helpful

1. How much do you think treatment can help your alcohol use?

Self-Stigma and Alcohol Dependence Scale [Source: Schomerus et al., 2011]
Please indicate the extent to which you agree or disagree with each of the following statements. There are no right or wrong answers.

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree

1. Because I have alcohol problems, I am unreliable.
2. Because I have alcohol problems, I am emotionally unstable
3. Because I have alcohol problems, I am violent
4. Because I have alcohol problems, I am living on other people's expenses
5. Because I have alcohol problems, I am self-pitying
6. Because I have alcohol problems, I am lazy
7. Because I have alcohol problems, I am resolving conflicts only with alcohol
8. Because I have alcohol problems, I am weak-willed
9. Because I have alcohol problems, I am unable to ever get away from alcohol
10. Because I have alcohol problems, I am unable to keep a regular job
11. Because I have alcohol problems, I am to blame for others' problems
12. Because I have alcohol problems, I am not to be trusted
13. Because I have alcohol problems, I am disgusting
14. Because I have alcohol problems, I am dirty and unkept
15. Because I have alcohol problems, I am below average intelligence
16. Because I have alcohol problems, I am unpredictable

Perceptions of Passing Down Propensity for Disease [source: adapted from Persky et al., 2015]

1. Do you have any biological children?
    a. If yes, how many _____
    b. If yes, what are their ages _____

2. Do you have any non-biological children (e.g., step-children)?
    a. If yes, how many _____
    b. If yes, what are their ages _____

Below are statements that some people have used to describe their feelings. Please read each statement and choose the most appropriate number to indicate how you feel about each statement. There are no right or wrong answers. Please give the answer which seems to describe your feelings best.

If you do not have children, please consider how you think you would respond if you did have children.

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree	Strongly agree

1. Alcohol problems are learned in the family

Genetics contributes to the development of alcohol problems

My children are at increased risk for developing alcohol problems because they are, in part, learned in the family

My children are at increased risk for developing alcohol problems because they are, in part, genetic

I feel guilty about the genetic risk for alcohol problems that I may have passed down to my child

I feel guilty about the genetic risk for overweight that I may have passed down to my child

I feel guilty about the behavioral risks for alcohol problems that my child may have learned from me

I feel guilty about the behavioral risks for overweight that my child may have learned from me

Sweet Preference Questionnaire Date:_

Timepoint:___

Listed below are questions that ask about your feelings about sweets. Please indicate how much you agree or disagree with each of the following statements by placing a single check mark (like this: X) along each line between STRONGLY DISAGREE and STRONGLY AGREE. The closer you place your check mark to one end or the other indicates the strength of your disagreement or agreement. Please complete every item.

1. Having something sweet to eat makes me feel less nervous or depressed.
   STRONGLY DISAGREE:_____:___:___:___:___:___:___:___:
   STRONGLY AGREE

2. I can easily control how many sweets I eat.

STRONGLY DISAGREE:_____:___:___:___:___:___:___:___: STRONGLY AGREE

3. I often crave something sweet when I'm nervous or depressed

STRONGLY DISAGREE:_____:___:___:___:___:___:___:___: STRONGLY AGREE

4. I would enjoy having something sweet to eat right now.

STRONGLY DISAGREE:_____:___:___:___:___:___:___:___: STRONGLY AGREE

5. Having something sweet to eat makes me feel happier.

STRONGLY DISAGREE:_____:___:___:___:___:___:___:___: STRONGLY AGREE

6. I often have an urge for something sweet to eat.

STRONGLY DISAGREE:_____:___:___:___:___:___:___:___: STRONGLY AGREE

7. I am less irritable if I have something sweet to eat.

STRONGLY DISAGREE:_____:___:___:___:___:___:___:___: STRONGLY AGREE

8. I have problems controlling how much sweet food I eat.

STRONGLY DISAGREE:_____:___:___:___:___:___:___: STRONGLY AGREE

9. I often feel guilty about how many sweets I eat.
STRONGLY DISAGREE:_____:___:___:___:___:___:___: STRONGLY AGREE

10. I have tried to cut down on eating sweets.
STRONGLY DISAGREE:_____:___:___:___:___:___:___: STRONGLY AGREE

11. I have found that eating sweets reduces my desire to drink alcohol
STRONGLY DISAGREE:_____:___:___:___:___:___:___: STRONGLY AGREE

12. I have used eating sweets to control how much alcohol I drink
STRONGLY DISAGREE:_____:___:___:___:___:___:___: STRONGLY AGREE

APPENDIX 8: NIAAA MRI Safety Questionnaire

Place Patient Label Here

Date: _____

NIAAA MRI SAFETY SCREENING QUESTIONNAIRE

Please circle each response:

Have you had any MRI scan at NIH within the last 12 months?.....…… yes no

*****Some of the following items may be hazardous to your safety and some can interfere with the MRI examination. Please select "yes" if you have any of the following or "no" if you do not*****

6. Have you ever worked with metal (grinding, fabrication, etc.) or ever had an injury to the eye involving a metallic object (e.g., metallic slivers, foreign body)?…… yes no
   If yes, please describe: _____
7. Have you ever had surgery or any similar invasive procedure?.....……. yes no
   If yes, list all prior surgeries and approximate dates (Use back side if necessary):
   _____
8. Women: Are you, or might you be, pregnant?.....…….. yes no
9. Women: Are you currently breast feeding?.....…. yes no
10. Do you have a history of kidney disease, seizure, diabetes, or any blood diseases (Anemia or Sickle Cell)?..... yes no
11. Do you have any drug or latex allergies?..... yes no
    If yes, please describe: _____
12. List your medications (Use back if necessary): _____
    _____
13. Have you ever had a reaction to a contrast medium used for MRI or CT?…….. yes no
14. Do you have claustrophobia (fear of closed places)?…….. yes no
15. Do you have any known hearing problems (e.g. ringing, sensitive to loud noise)?..... yes no
16. Cannot lay on your back comfortably for an extended period of time up to 2 hours?..... yes no

12. Do you have any of the following:

Cardiac pacemaker……	yes no	Any type of prosthesis (eye, penile)...	yes no
Implanted cardiac defibrillator……	yes no	Heart valve prosthesis…….	yes no
Aneurysm clip……	yes no	Shunt (spinal/intraventricular)…….	yes no
Neuro or Bone Stimulator……	yes no	Wire sutures or surgical staples……..	yes no
Insulin or infusion Pump……..	yes no	Bone/joint pin, screw, nail, plate……	yes no
Implanted drug infusion device……..	yes no	Body tattoos or makeup (eyeliner/lip)	yes no
Cochlear, otologic, or ear implant…..	yes no	Body piercing(s) (non-removable)…..	yes no
Prostate radiation seeds……..	yes no	Breast tissue expander…….	yes no
IUD (intrauterine device)……	yes no	Any metallic implants or objects…….	yes no
Transdermal medication patch (Nitro)	yes no		

Patient (legal guardian) signature _____ Date_____

Researcher signature _____ Date _____

If any of the above items (1-12) is answered "yes", the MRI scan must be verified by a physician or a nurse practitioner responsible for the scan.

Physician signature _____ Date:_____

Scan ☐ Approved ☐ Not Approved, Reason: _____

Appendix 9

Alcohol Urge Questionnaire (AUQ)

Affix Patient Label Here

Alcohol Urge Questionnaire (AUQ)

Date:_____

Timepoint:_____

Listed below are questions that ask about your feelings about drinking. The words "drinking" and "have a drink" refer to having a drink containing alcohol, such as beer, wine or liquor.

Please indicate how much you agree or disagree with each of the following statements by placing a single mark (like this: X) along each line between STRONGLY AGREE and STRONGLY DISAGREE. The closer you place your mark to one end or the other indicates the strength of your disagreement or agreement. Please complete every item. We are interested in how you are thinking or feeling *right now* as you are filling out the questionnaire.

RIGHT NOW

1. All I want to do now is have a drink.

   STRONGLY DISAGREE:_____:_____:_____:_____:_____:_____:_____: STRONGLY AGREE

2. I do not need to have a drink now.

   STRONGLY DISAGREE:_____:_____:_____:_____:_____:_____:_____: STRONGLY AGREE

3. It would be difficult to turn down a drink this minute.

   STRONGLY DISAGREE:_____:_____:_____:_____:_____:_____:_____: STRONGLY AGREE

4. Having a drink now would make things seem perfect.

   STRONGLY DISAGREE:_____:_____:_____:_____:_____:_____:_____: STRONGLY AGREE

5. I want a drink so bad I can almost taste it.

   STRONGLY DISAGREE:_____:_____:_____:_____:_____:_____:_____: STRONGLY AGREE

6. Nothing would be better than having a drink right now.

   STRONGLY DISAGREE:_____:_____:_____:_____:_____:_____:_____: STRONGLY AGREE

7. If I had the chance to have a drink, I don't think I would drink it.

   STRONGLY DISAGREE:_____:_____:_____:_____:_____:_____:_____: STRONGLY AGREE

8. I crave a drink right now.

   STRONGLY DISAGREE:_____:_____:_____:_____:_____:_____:_____: STRONGLY AGREE

Appendix 10:**Mindful Attention Awareness Scale**

Day-to-Day Experiences

Instructions: Below is a collection of statements about your everyday experience. Using the 1-6 scale below, please indicate how frequently or infrequently you currently have each experience. Please answer according to what *really reflects* your experience rather than what you think your experience should be. Please treat each item separately from every other item.

1	2	3	4	5	6
Almost Always	Very Frequently	Somewhat Frequently	Somewhat Infrequently	Very Infrequently	Almost Never

I could be experiencing some emotion and not be conscious of it until some time later.	1	2	3	4	5	6
I break or spill things because of carelessness, not paying attention, or thinking of something else.	1	2	3	4	5	6
I find it difficult to stay focused on what's happening in the present.	1	2	3	4	5	6
I tend to walk quickly to get where I'm going without paying attention to what I experience along the way.	1	2	3	4	5	6
I tend not to notice feelings of physical tension or discomfort until they really grab my attention.	1	2	3	4	5	6
I forget a person's name almost as soon as I've been told it for the first time.	1	2	3	4	5	6
It seems I am "running on automatic," without much awareness of what I'm doing.	1	2	3	4	5	6
I rush through activities without being really attentive to them.	1	2	3	4	5	6
I get so focused on the goal I want to achieve that I lose touch with what I'm doing right now to get there.	1	2	3	4	5	6
I do jobs or tasks automatically, without being aware of what I'm doing.	1	2	3	4	5	6
I find myself listening to someone with one ear, doing something else at the same time.	1	2	3	4	5	6
I drive places on 'automatic pilot' and then wonder why I went there.	1	2	3	4	5	6
I find myself preoccupied with the future or the past.	1	2	3	4	5	6
I find myself doing things without paying attention.	1	2	3	4	5	6
I snack without being aware that I'm eating.	1	2	3	4	5	6

Scoring: To score the scale, simply compute a mean (average) of the 15 items. Higher scores reflect higher levels of dispositional mindfulness.

Curiosity and Exploration Inventory-II

Curiosity and Exploration Inventory (CEI-II) *Instructions*: Rate the statements below for how accurately they reflect the way you generally feel and behave. Do not rate what you think you should do, or wish you do, or things you no longer do. Please be as honest as possible.		Very Slightly or Not at all	A Little	Moderately	Quite a Bit	Extremely
1.	I actively seek as much information as I can in new situations.	1	2	3	4	5
2.	I am the type of person who really enjoys the uncertainty of everyday life.	1	2	3	4	5
3.	I am at my best when doing something that is complex or challenging.	1	2	3	4	5
4.	Everywhere I go, I am out looking for new things or experiences.	1	2	3	4	5
5.	I view challenging situations as an opportunity to grow and learn.	1	2	3	4	5
6.	I like to do things that are a little frightening.	1	2	3	4	5
7.	I am always looking for experiences that challenge how I think about myself and the world.	1	2	3	4	5
8.	I prefer jobs that are excitingly unpredictable.	1	2	3	4	5
9.	I frequently seek out opportunities to challenge myself and grow as a person.	1	2	3	4	5
10.	I am the kind of person who embraces unfamiliar people, events, and places.	1	2	3	4	5
Stretching: 1, 3, 5, 7 / Embracing: 2, 4, 6, 8, 10.						

University Rhode Island Change Assessment Scale (URICA)

Each statement below describes how a person might feel when stating therapy or approaching problems in his life. Please indicate the extent to which you tend to agree or disagree with each statement. In each case, make you choice in terms of how you feel right now, not what you have felt in the past or would like to feel. For all the statements that refer to your "problem", answer in terms of problems related to substance use and/or mental health. The words, "here" and "this place" refer to your treatment center.

There are five possible responses to each of the items in the questionnaire:

1=Strongly Disagree
2=Disagree
3=Undecided
4=Agree
5=Strongly Disagree

Circle the number that best describes how much you agree or disagree with each statement.

	Strongly Disagree	Disagree	Undecided	Agree	Strongly Agree
1, As far as I am concerned, I don't have any problems that need changing.	1	2	3	4	5
2. I think I might be ready for some self-improvement.	1	2	3	4	5
3. I am doing something about the problems that had been bothering me.	1	2	3	4	5
4. It might be worthwhile to work on my problem.	1	2	3	4	5
5. As far as I am concerned, I don't have any problems that need chaning	1	2	3	4	5
6. I am not the problem one. It doesn't make much sense for me to consider changing.	1	2	3	4	5
7. I am finally doing some work on my problem.	1	2	3	4	5
8. I have been thinking that I might want to change something about myself.	1	2	3	4	5
9. I have been successful in working on my problem, but I'm not sure I can keep up the effort on my own.	1	2	3	4	5
10. At times my problem is difficult, but I am working on it.	1	2	3	4	5
11. Trying to change is pretty much a waste of time for me because the problem doesn't have to do with me.	1	2	3	4	5
12. I'm hoping that I will be able to understand	1	2	3	4	5

myself better.					
13. I guess I have faults, but there is nothing that I really need to change.	1	2	3	4	5
14. I am really working hard to change.	1	2	3	4	5
15. I have a problem, and I really think I should work on it.	1	2	3	4	5
16. I'm not following through with what I had already changed as well as I had hoped, and I want to prevent a relapse of the problem.	1	2	3	4	5
17. Even though I'm not always successful in changing, I am at least working on my problem.	1	2	3	4	5
18. I thought once I had resolved the problem I would be free of it, but sometimes I still find myself struggling with it.	1	2	3	4	5
19. I wish I had more ideas on how to solve my problems.	1	2	3	4	5
20. I have started working on my problem, but I would like help.	1	2	3	4	5
21. Maybe someone or something will be able to help me.	1	2	3	4	5
22. I may need a boost right now to help me maintain the changes I've already made.	1	2	3	4	5
23. I may be part of the problem, but I don't really think I am.	1	2	3	4	5
24. I hope that someone will have some good advice for me.	1	2	3	4	5
25. Anyone can talk about changing; I'm actually doing something about it.	1	2	3	4	5
26. All this talk about psychology is boring. Why can't people just forget about their problems.	1	2	3	4	5
27. I'm struggling to improve myself from having a relapse of my problem.	1	2	3	4	5
28. It is frustrating, but I feel I might be having a recurrence of a problem I thought I had resolved.	1	2	3	4	5
29. I have worries, but so does the next guy.	1	2	3	4	5

30. I am actively working on my problem.	1	2	3	4	5
31. I would rather cope with my faults than try to change them.	1	2	3	4	5
32. After all I had done to try to change my problem, every now and again it comes back to haunt me.	1	2	3	4	5

URICA Scoring

1. Obtain the average score per subscale using the following grid:

Pre-contemplation (PC)	Contemplation (C)	Action (A)	Maintenance (M)
1._____	2._____	3._____	6._____
5._____	8._____	7._____	16._____
11._____	12._____	10._____	18._____
13._____	15._____	14._____	22._____
23._____	19._____	17._____	27._____
26._____	21._____	25._____	28._____
29._____	24._____	30._____	32._____
Total_____ divided by 7= Average	Total_____ divided by 7= Average	Total_____ divided by 7= Average	Total_____ divided by 7= Average

2. Compute the "Readiness for Change" score via the following formula:

   (Avg C + Avg A + Avg M) – Avg PC

3. Compare the Readiness for Change score to the following group means. Choose the stage whose group average is closest to the computed Readiness Score:

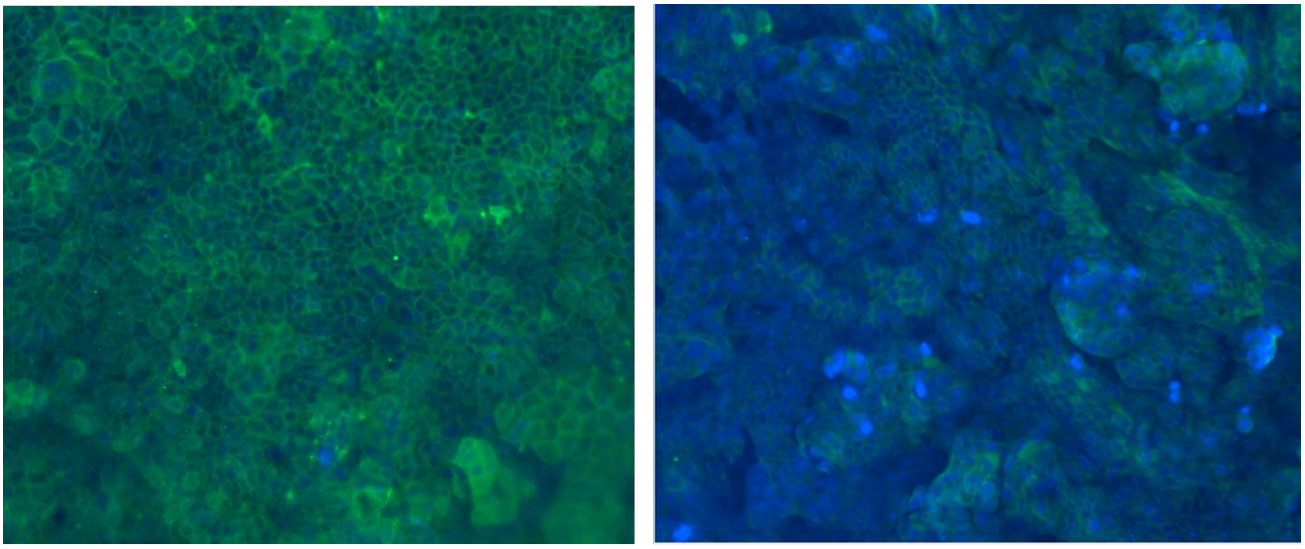
STAGE	GROUP AVG
Pre-Contemplation	9.3
Contemplation	11.0
Participation (Action)	12.6
Maintenance	(Not Available)

Appendix 11

Lack of transport of PF-5190457 by P-glycoprotein

Armin Sadighi and Fatemeh Akhlaghi, Clinical Pharmacokinetics Research Laboratory, University of Rhode Island

The Pfizer original investigator's brochure indicate that PF-5190457 is transported by P-glycoprotein 1 (permeability glycoprotein, abbreviated as P-gp or Pgp) also known as multidrug resistance protein 1 (MDR1). The basis of their experiments was in MDCK cell line and P-gp knockout mice. To establish whether or not PF-5190457 is transported by human P-gp, we have conducted in vitro transport experiments in normal Caco-2 cells and Caco-2 cells over expressing P-glycoprotein. Caco-2 cells was derived from colon of human Caucasian colon adenocarcinoma. In order to induce P-gp, Caco-2 cells were treated with an anticancer agent, vinblastine until a resistance strain of the cells were developed. Immunohistochemistry of vinblastine treated cells is shown in Figure 1 were green florescence indicate the increased expression of P-gp.



P-gp induced Caco-2 cells, PN 56 **Normal Caco-2 cells, PN 56**

Figure 1. The higher expression of P-gp transporter on the membrane of P-gp induced cells compare to partial expression of the same transporter in normal Caco-2 cells has been confirmed using immunofluorescent imaging of cells. (Magnification ×20).

The vinblastine treated cells were then functionally evaluated by the use of Calcine AM assay. This assay is a well-established test for evaluating the functional activity of P-gp. The P-gp activity in induced cells were 2-fold higher than normal Caco-2 cells (Figure 2). Inhibitors of P-gp (verapamil and cyclosporine) reduced florescence intensity of calcine AM assay in a dose dependent manner while these inhibitors had no influence on normal Caco-2 cells (Figure 2).

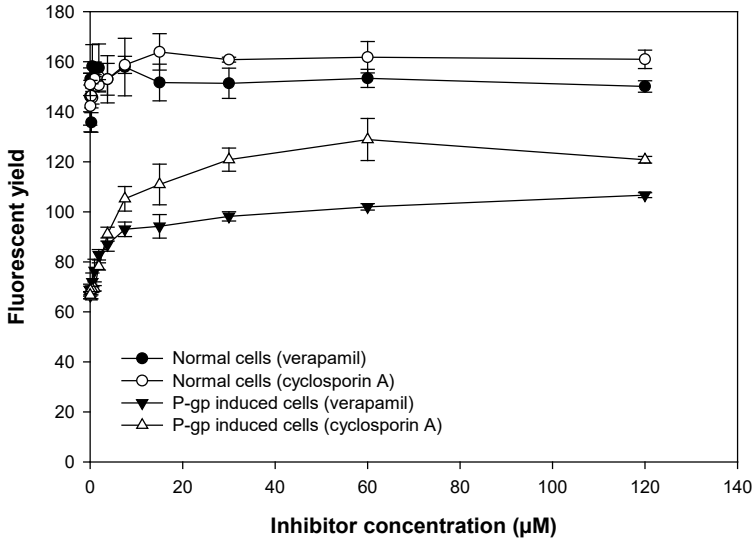


Figure 2. The fluorescent yield of calcein measured for normal and P-gp induced Caco-2 cells in the presence of verapamil and cyclosporine A (P-gp inhibitors). Results show that by increasing the concentration of inhibitor in P-gp induced cells the fluorescent yield was increased, while there is no significant change for normal cells. The plot shows that the functional activity of P-gp at baseline (no inhibitor present) in transporter-induced cells was more than 2 times higher than that in normal cell line.

Then transport studies of PF-5190457 was carried out in both normal and P-gp induced cells lines. PF-5190457 transport across P-gp induced Caco-2 cells was carried out by adding the drug (5 μg/mL) to apical (A) and basolateral (B) sides of normal and P-gp induced cells. The PF-57 transport was done for 4 h and results obtained from three independent tests and reported as Mean±SD. UR and ER were calculated <2 which means that PF-5190457 neither is actively uptaked nor effluxed by P-gp (Table 1).

Table 1. transport of PF-5190457 in normal Caco-2 and P-gp induced cells.

Parameters	Normal cells	
	A-B	B-A
Flux	0.07±0.01	0.03±0.001
P_{app} ($\times 10^{-6}$ cm/s)	3.74±0.31	4.36±0.28
UR	0.86±0.04	
ER	1.17±0.05	

Parameters	P-gp induced cells	
	A-B	B-A
Flux	0.06±0.01	0.02±0.001
P_{app} ($\times 10^{-6}$ cm/s)	3.53±0.76	3.52±0.14
UR	1.02±0.26	
ER	1.01±0.25	

We have then investigated the effect of ethanol exposure on the possible transport of PF-5190457 by P-gp. PF-5190457 transport across P-gp induced Caco-2 cells was carried out by adding the drug (5 µg/mL) to apical (A) and basolateral (B) sides of cells. The drug transport was assessed after 24 h treatment in the absence (0 mM) and presence of EtOH (25-500 mM). Cells were kept in the saturated EtOH chambers with the corresponding EtOH concentration at the humidified incubator (37 °C, 95% air+5% CO_2) and by the end of 1 day treatment, the PF-5190457 transport experiments were performed for 4 h.

The results obtained from three independent tests and reported as Mean ± SD. Statistical significance between mean values was calculated using ANOVA (Sigma plot version 12). Probability values <0.05 were considered significant. Table 2 shows the result of this experiment. No statistically significant difference was observed for B-A transport of PF-5190457 in the absence and presence of 200 µl verapamil (P-gp inhibitor) for EtOH treated cells compare to negative control. Based on the results, while Efflux ration <2 of PF-5190457 indicated that this compound is not a P-gp substrate.

Table 2. PF-5190457 transport across P-gp induced Caco-2 cells in the presence of different EtOH concentrations

Parameters	EtOH concentration (mM)	A-B		B-A	
		Without Verapamil	With Verapamil	Without Verapamil	With Verapamil
P_{app} ($\times 10^{-6}$ cm/s)	0	0.82±0.08*	0.34±0.06**	0.49±0.03***	0.32±0.08****
	25	1.00±0.33	0.81±0.29	0.5±0.15	0.41±0.02
	50	1.28±0.37	1.16±0.05	0.42±0.07	0.37±0.06
	100	1.59±0.08	1.45±0.25	0.37±0.06	0.45±0.05
	500	1.50±0.08*	1.28±0.01**	0.52±0.06***	0.38±0.15****
UR	0	1.69±0.27	1.17±0.58		
	25	2.08±0.86	2±0.64		
	50	3.06±0.66	3.48±0.11		
	100	4.4±0.75	3.13±0.19		
	500	2.9±0.15	4.6±2.32		
ER	0	0.6±0.1	0.98±0.49		
	25	0.53±0.18	0.53±0.17		
	50	0.34±0.07	0.29±0.01		
	100	0.23±0.04	0.32±0.02		
	500	0.35±0.02	0.25±0.13		

* P-value: 0.01, **P-value: 0.079, *** P-value: 0.22, **** P-value: 0.5

Appendix 12 Standard Operation Procedures

Standard of Procedure Sweet Preference

Sweet Preference Task Setup

Script:

We will do the sweet preference task. We do this with our subjects to determine two things: whether they perceive things to be sweet and whether they are a "sweet liker". You will be presented with 25 small cups of different solutions. I will hand you one at a time and you will swish each solution in your mouth for 5 seconds and then spit it out. After swishing the solution, you will then answer two questions on the computer about that solution you just tasted. You will then take a sip of water, swish and spit that out as well. You will then repeat this 25 times total. Remember not to swallow the solutions or the water.

ITEMS NEEDED:

1. Mini Cups
2. Tray
3. Permanent Marker
4. Pink Basin
5. SPT Solutions (5 vials of 5 solutions, 25 vials total)
6. Water

PROCEDURE:

Retrieve SPT Solutions from Nursing Station refrigerator

1. Take out 25 mini cups, label them 1-25
2. Lay out cups in 5 rows of 5 columns on the tray like below (with 1,6,11,16,21 facing closest to participant):

1	6	11	16	21	← A solutions
2	7	12	17	22	← B solutions
3	8	13	18	23	← C solutions
4	9	14	19	24	← D solutions
5	10	15	20	25	← E solutions

3. Open the bag of “A” solutions and fill the first row of cups
4. Open the bag of “B” solutions and fill the second row of cups
5. Open the bag of “C” solutions and fill the third row of cups
6. Open the bag of “D” solutions and fill the fourth row of cups
7. Open the bag of “E” solutions and fill the fifth row of cups
8. Set up SPT Kiosk on computer for subject
9. Have subject swish Solution #1 for 5 seconds and spit into basin
10. Have subject complete SPT Kiosk for Trial #1 (two questions per trial)
11. Have subject swish water for 5 seconds and spit into basin
12. Repeat steps 9 through 11 for Solutions #2-25

Appendix 13

Snaith Hamilton Pleasure Scale (SHAPS)

Name:

Date:

Snaith Hamilton Pleasure Scale (SHAPS)

This questionnaire is designed to measure your ability to experience pleasure *in the last week.*

It is important to read each statement very *carefully*.

Mark *one* of the boxes [ ] to indicated how much you agree or disagree with each statement.

1. I would enjoy my favorite television or radio program:

   Strongly disagree [ ]

   Disagree [ ]

   Agree [ ]

   Strongly agree [ ]

2. I would enjoy being with my family or close friends:

   Definitely Agree[ ]

   Agree [ ]

   Disagree [ ]

   Strongly disagree [ ]

3. I would find pleasure in my hobbies and pastimes:

   Strongly disagree [ ]

Disagree [ ]

Agree [ ]

Strongly agree [ ]

4. I would be able to enjoy my favorite meal:

Definitely Agree[ ]

Agree [ ]

Disagree [ ]

Strongly disagree [ ]

5. I would enjoy a warm bath or refreshing shower:

Definitely Agree[ ]

Agree [ ]

Disagree [ ]

Strongly disagree [ ]

6. I would find pleasure in the scent of flowers or the smell of a fresh sea breeze or freshly baked bread:

   Strongly disagree [ ]

   Disagree [ ]

   Agree [ ]

   Strongly agree [ ]

7. I would enjoy seeing other people's smiling faces:

   Definitely Agree[ ]

   Agree [ ]

   Disagree [ ]

   Strongly disagree [ ]

8. I would enjoy looking smart when I have made an effort with my appearance:

   Strongly disagree [ ]

   Disagree [ ]

   Agree [ ]

   Strongly agree [ ]

9. I would enjoy reading a book, magazine or newspaper:

   Definitely Agree[ ]

   Agree [ ]

Disagree [ ]

Strongly disagree [ ]

10. I would enjoy a cup of tea or coffee or my favorite drink:

Strongly disagree [ ]

Disagree [ ]

Agree [ ]

Strongly agree [ ]

11. I would find pleasure in small things, e.g. bright sunny day, a telephone call from a friend:

Strongly disagree [ ]

Disagree [ ]

Agree [ ]

Strongly agree [ ]

12. I would be able to enjoy a beautiful landscape or view:

Definitely Agree[ ]

Agree [ ]

Disagree [ ]

Strongly disagree [ ]

13. I would get pleasure from helping others:

Strongly disagree [ ]

Disagree [ ]

Agree [ ]

Strongly agree [ ]

14. I would feel pleasure when I receive praise from other people:

Definitely Agree[ ]

Agree [ ]

Disagree [ ]

Strongly disagree [ ]