Pharmacogenetic Study of Ondansetron in Alcohol Use Disorder

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A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Phase 2 Pharmacogenetic Study of Ondansetron in Alcohol Use Disorder

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Study Summary

Title	A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Phase 2 Pharmacogenetic Study of Ondansetron in Alcohol Use Disorder
Short Title	Ondansetron for Alcohol Use Disorder (AUD)
Protocol Number	HP 00061575
Phase	Phase 2
Methodology	This is a randomized, two-center, double-blind, parallel-group, placebo-controlled study. Comparable, but not identical studies will be conducted at the two sites
Study Duration	*5 years*
Study Center(s)	University of Pennsylvania (Penn) and University of Maryland Baltimore (UMB)
Objectives	**Primary specific aim**: to test the hypothesis that alcohol-dependent individuals of European ancestry (EAs) or of African American ancestry (AAs) carrying **any one or a combination** of specific genotypes will have a significantly lower number of drinks/drinking day (DDD) in response to ondansetron 0.33 mg twice daily, compared to those who are not carrying any of the selected genotypes or those who were treated with the placebo. **Secondary exploratory aim)**: We further hypothesize that specific genotypes (which differ for AAs and EAs) will moderate the response to ondansetron. The genotypes of interest reflect polymorphisms in the genes encoding the serotonin transporter (*SLC6A4*) and the 5-HT3A (*HTR3A*) and 5-HT3B (*HTR3B*) receptor subunits. We also will test the additional secondary predictions that the differences seen in our primary specific aim (above) will extend to secondary drinking measures and measures of quality of life. Additionally, we will use genome-wide expression studies of total RNA and miRNA- from blood samples and supported by supplemental funding-to compare the 15 most responsive subjects (whose drinking is reduced most significantly) and the 15 least responsive subjects on DDD and the 15 subjects with the greatest number of adverse events and the 15 with the fewest to identify changes that mediate ondansetron's efficacy and adverse events profile, respectively.
Number of Subjects	366 at two sites (183 at each site)
Diagnosis and Main Inclusion Criteria	Current DSM-5 diagnosis of Alcohol Use Disorder; AUDIT score ≥ 8; >6 Heavy Drinking Days (HDDs)- define as a day with alcohol consumption of ≥5 standard drinks for men, and ≥4 standard drinks for women in the last 28 days; currently drinking an average of ≥14 standard drinks/week (women) and ≥21 standard drinks/week (men) in the last 28 days (and have met these criteria for the 7 days prior to randomization); and expressed a wish to reduce or stop drinking.
Study Product, Dose, Route, Regimen	Ondansetron 0.33 mg or matching placebo by mouth twice daily.
Duration of administration	16weeks
Reference therapy	Placebo

Statistical Methodology	All randomized subjects will be included in the analysis using the intent-to-treat principle. We will use a mixed-models approach, adjusting for baseline covariates (when significantly different by group) and the same drinking variables for baseline. For the drinking variables, the baseline value is the average for the 30-day period prior to the screening visit. Other potential covariates shall include age, race, and sex. The primary outcome measure will be DDD during the 16-week treatment period.

1 Introduction

This document is a protocol for a human research study. This clinical trial will be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and institutional research policies and procedures.

1.1 Background

Alcohol use disorder (AUD) is a heterogeneous and chronic relapsing disorder that includes both acute (binge drinking) and chronic (frequent heavy drinking) dimensions. Globally and in the United States, AUD ranks 5th and 3rd, respectively, among the most common preventable causes of morbidity and mortality.

Approximately 7.2 percent or 17 million adults in the United States ages 18 and older had an AUD in 2012. This includes 11.2 million men and 5.7 million women. AUD can cause serious health, family, and economic problems. Finding treatments that are effective in decreasing heavy drinking among individuals with AUD is, therefore, an important scientific and health goal. Only a fraction of the people who could benefit from AUD treatment receives treatment. In 2012, for example, 1.4 million adults received treatment for an AUD at a specialized facility (8.4 percent of adults in need).

A novel and important strategy to improve AUD treatment outcomes uses a personalized medicine approach to optimize treatment response. This approach matches a subject with specific genotypes to an appropriate medication with a high probability of a positive treatment outcome while also potentially minimizing adverse events. Despite the development of new medications to treat AUD, pharmacotherapy remains an underutilized approach. This is, in large measure, a function of modest effect sizes associated with the FDA-approved medications for AUD.

Personalized medicine offers the prospect of greater efficacy and safety by matching subjects with optimal treatments.

This proposed study will address important limitations in a previously published Phase II study (Johnson et al. 2011) conducted in a sample of European Americans (EA). In the first study12, Johnson et al. reported that 5-HTTLPR:LL genotype in the gene that encodes the serotonin transporter molecule (SLC6A4) either alone or in combination with rs1042173:TT of the same gene predicted response to ondansetron. In a secondary analysis of the same sample, the authors reported three additional genotypes in the two genes that encode the primary binding site of ondansetron – the 5-HT3 sub unit genes HTR3A and HTR3B1. As the primary aim of this study, we will test the replicability of these findings with following modifications to address several limitations of the prior studies. First, we will stratify the population into two main groups of participants based on whether a participant is carrying any of the responsive genotypes (Genotype responsive group vs. Genotype non-responsive group). There are three main differences between the Genotype-responsive group defined by Johnson et al 2013 and the present study.

(1) In this study, we will use the tri-allelic classification for 5-HTTLPR of SLC6A4, the gene encoding the serotonin transporter (LA, LG or S alleles) to avoid the underestimation of pharmacogenetic effects associated with the use of the bi-allelic classification (L or S alleles).

We will limit the LL group to include only those who are LALA, will be considered as Genotype-responsive as the LG allele has been shown to be associated with lower expression levels of 5-HTT mRNA similar to the S allele (2).
(2) Carriers of rs1042173:TT (without 5-HTTLPR:LALA) will be included in the Genotype responsive group, based on the findings that indicated a significant risk effect of rs1042173:TT on heavy drinking3.
(3) Treatment assignment within Genotype Responsive group will be balanced at randomization according to genotype subgroups listed below, to accurately characterize the degree to which ondansetron's therapeutic effect is enhanced by either one of SLC6A4 or any of the HTR3A/B genotypes.
a. LALA in the absence of rs1042173:TT and regardless of presence/absence of HTR3A/B genotypes
b. rs1042173:TT in the absence of LALA and regardless of presence/absence of HTR3A/B genotypes
c. LALA together with rs1042173:TT and regardless of presence/absence of HTR3A/B genotypes
d. Any one or more HTR3A/B genotypes in the absence of both LALA and rs1042173:TT
Number of participants in the two main groups (Genotype responsive vs. non-responsive) as well as the four subgroups will represent population frequencies of constituent genotypes. This approach, as opposed to having equal numbers in each group, was adopted in order to include all participants who are otherwise qualified to be enrolled in this study. At the UMB site, we will enroll both African Americans (AAs) and EAs. Because of differences in population frequencies and linkage disequilibrium structures, in AAs, we will explore the moderating effect of a non-synonymous, functional SNP (rs1176744) that has been shown to be associated with alcohol dependence in AAs (Enoch et al. 2011). This SNP is in *HTR3B*, a gene in which variation was shown in the published Phase II study (Johnson et al. 2013) to moderate the response to ondansetron. (3) We will use the tri-allelic classification for 5-HTTLPR of *SLC6A4*, the gene encoding the serotonin transporter (L_A, L_G or S alleles) to avoid the underestimation of pharmacogenetic effects associated with the use of the bi-allelic classification (L or S alleles). We will limit the LL group to include only those who are L_AL_A, as the L_G allele has been shown to be associated with lower expression levels of 5-HTT mRNA similar to the S allele and will balance individuals within genotype (L_AL_A vs. non- L_AL_A between the ondansetron and placebo groups in the primary randomization (4) We will also balance individuals in the primary randomization within the two genotype groups of the *SLC6A4* 3′-UTR SNP rs1042173 (TT vs TG/GG). This will make it possible to characterize accurately the degree to which ondansetron's therapeutic effect is moderated by rs1042173 and determine the independent therapeutic effect of ondansetron in individuals with the TT genotype. Finally, we will examine mRNA expression in a subset of individuals from the study to test whether the observed moderating effects of genotype are evident also in the expression of *HTR3B* and *SLC6A4*.

In addition to conducting analyses separately using genotypes that are relevant to each racial group, we will examine the findings from the two sites in a joint analysis.

1.2 Investigational Agent

Ondansetron, a specific serotonin-3 (5-HT_3) antagonist is FDA approved to treat chemotherapy-induced nausea and vomiting, radiation-induced nausea and vomiting, and postoperative nausea and vomiting. In the present study, subjects will be given ondansetron 0.33 mg or matching placebo capsules twice daily.

The active ingredient in ondansetron tablets is ondansetron hydrochloride (HCl) as the dihydrate, the racemic form of ondansetron. Chemically it is (±) 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one, monohydrochloride, dihydrate. Ondansetron's empirical formula is C18H19N3O•HCl•2H2O, representing a molecular weight of 365.9.

1.3 Preclinical Data

Ondansetron's effects are mediated through 5-HT_3 receptors in the brain. Transgenic mice over-expressing 5-HT_3 receptors in the forebrain have been used to study alterations in 5-HT current induced by ethanol within the brain (Engel & Allan, 1999; Engel, Lyons, & Allan, 1998; Sung, Engel, Allan, & Lovinger, 2000). These studies have shown that 5-HT_3 over-expression reduced ethanol consumption, improved contextual fear conditioning, reduced anxiety, increased inspective behavior toward a novel object and increased dopamine release in the nucleus accumbens (Allan, Galindo, Chynoweth, Engel, & Savage, 2001; Engel & Allan, 1999; Engel, Lyons, & Allan, 1998; Sung, Engel, Allan, & Lovinger, 2000; Metz, Chynoweth, & Allen; 2006). Using a transgenic mouse model lacking the gene for the 5-HT_{3A} receptor subunit, Hodge and colleagues (2004) showed that the reduction of alcohol drinking produced by 5-HT_3 antagonism is dependent on the presence of 5-HT_{3A} subunit-containing receptor complexes. While these data, especially the directional effect, cannot be extrapolated directly to studies in wild-type animals that can differ in the density and neuroanatomical distribution of 5-HT_3 receptors or to human studies, they do provide a framework which suggests that differential 5-HT_3 expression can influence alcohol consumption.

1.4 Clinical Data to Date

Serotonin (5-HT) function is an important regulator of alcohol consumption. The serotonin transporter (5-HTT), which is encoded by the gene *SLC6A4*, is the major modulator of neuronal 5-HT function. Variation in *SLC6A4* has been associated in humans with differences in alcohol craving and consumption. Specifically, in some studies, alcohol-dependent individuals who are homozygous for the long (L) allele of an insertion deletion polymorphism in the 5′-regulatory region of *SLC6A4* (5-HTTLPR) or the T allele of the single nucleotide polymorphism (SNP) rs1042173 in the 3′-untranslated region (3′-UTR) of that gene report increased craving and consumption of alcohol.

SLC6A4, which is located on chromosome 17q11.1–q12, is the only known gene encoding the serotonin transporter (5-HTT) in the human genome The *SLC6A4* promoter contains a functional insertion-deletion polymorphism (5′-regulatory region of the 5-HTT; 5-HTTLPR) that results in a long (L) form, which possesses 44 base pairs that are absent in the short (S) allele. The 5-HTTLPR polymorphism has been investigated extensively for a possible association with a wide range of psychiatric disorders including alcohol dependence. Recent research suggests a gene-by-environment interaction. A neuroimaging study showed that alcohol-dependent LL genotype individuals had lower binding of the radioligand ^{123}β-CIT (2 beta-carbomethoxy-3 beta-(4-iodophenyl) tropane) to the 5-HTT in the raphe nuclei of the brain than did healthy LL genotype individuals (Heinz et al., 2000). In contrast, within S-carriers 5-HTT availability did not differ significantly between control and alcoholic subjects. Previous studies conducted by our own research team have also shown similar patterns of 5-HTTLPR genotype-based differences in 5-HTT availability and function. Specifically, among individuals with the L allele, a greater quantity of lifetime alcohol consumption was associated with lower levels of 5-HT uptake and binding (Javors et al., 2005; Johnson et al., 2008). Taken together, these findings suggest a potential selective susceptibility of L-allele carrying individuals to the neurotoxic effects of chronic excessive alcohol consumption. Johnson et al. (2013) discovered that another functional allelic variant, the SNP rs1042173 (T/G) in the 3′- untranslated region (3′-UTR) of *SLC6A4*, is associated with the intensity of alcohol consumption measured by the

number of standard drinks (1 standard drink = about 14g of 100% alcohol) consumed per drinking day (Seneviratne, Huang, Ait-Daoud, & Johnson, 2009). TT homozygotes at this locus, compared with G-allele carriers, had a significantly greater intensity of alcohol consumption. T-allele-transfected HeLa cells had lower levels than their G allele counterparts of expression of both 5-HTT mRNA and protein. Because rs1042173 is located at or near a potential binding site for several microRNAs in the 3′-UTR of the gene, the variant may alter expression levels by reducing the stability of mRNA.

Lowered 5-HTT expression would, paradoxically, be associated with reduced rather than increased 5-HT synaptic neurotransmission and consequent upregulation of postsynaptic 5-HT receptors. This is because the 5-HTT in the raphe nuclei is somatodendritic, and, therefore, negative feedback mechanisms would decrease 5-HT neuron firing rates and upregulate postsynaptic 5-HT receptors. In sum, these findings suggest that blockade of upregulated 5-HT_3 receptors by ondansetron (a specific 5-HT_3 receptor antagonist) would reduce heavy drinking. We hypothesize that, among individuals with an AUD, the alcohol consumption-promoting effects of the 5-HTTLPR and 3′-UTR variants are due to lower 5-HTT function and decreased 5-HTT expression through reduced stability of mRNA, respectively—effects that individually lead to substantial postsynaptic 5-HT upregulation and, when combined, a supra-additive effect.

In a phase 2, randomized, double-blind clinical trial of 283 alcohol-dependent individuals of EA or AA ancestry, treatment with the specific serotonin-3 (5-HT_3) antagonist ondansetron, presumably through its neuromodulatory effects at postsynaptic 5-HT receptors, significantly reduced drinking among those who were homozygous for the L allele of the 5-HTTLPR—an effect that was enhanced in individuals who were T-allele homozygotes for the 3′-UTR SNP (Johnson et al., 2011). Further analysis of this data set revealed additional polymorphisms located on the genes for the A and B subunits of the serotonin 5-HT_3 receptor that were also associated with a therapeutic response to ondansetron (Johnson, Seneviratne, Wang, Ait-Daoud, & Li, 2013

The proposed study is designed to address several important limitations of the above mentioned phase 2 clinical trials that tested the efficacy of ondansetron in AUD. Addressing these limitations (listed below), would further establish the use of ondansetron as an efficacious pharmacotherapy for AUD utilizing a personalized genetic (pharmacogenetic) approach. The current study is a proof-of-concept, double-blind, randomized clinical trial to test whether, treatment-seeking, alcohol-dependent AA and EA adults carrying specific genotypes show a significantly greater and more robust treatment response to ondansetron than placebo and/or non-carriers of the genotypes, as reflected in greater reductions in DDD.

Genetic association studies: An examination of 360 treatment-seeking AA male subjects with DSM-IV lifetime diagnoses of alcohol, cocaine, and heroin dependence and 187 showed a significant association between alcohol dependence and the functional SNP rs1176744 in *HTR3B* which encodes a subunit of the 5-HT_{3AB} receptor (Enoch et al., 2011). These findings were supported by earlier work from the same group that showed an association between a 5-HT_{3B} genotype and alcohol dependence with antisocial behaviors (Ducci et al., 2009). A recent candidate gene association analysis in EAs also showed a strong interactive effect of 5-HTTLPR, rs1042173 and 5-HT_{3AB} variants associated with AUD (Seneviratne et al., 2013). Taken together, these studies suggest that variation in the genes encoding the 5-HT3 receptor and serotonin transporter may contribute to the development of alcoholism and lay the foundation for an examination of how certain polymorphic variants in the genes may differentially influence response to ondansetron treatment. Nevertheless, because polymorphic variants associated with alcoholism may differ from those that moderate the ondansetron treatment response, effects of polymorphisms on treatment response will have to be demonstrated in a clinical study of ondansetron, rather than only in disease risk association studies.

Clinical Study: Based on the expectation that allelic variants within either homomeric or heteromeric 5-HT_3 receptors could impact the response to ondansetron, Johnson et al. (2011) conducted an additional analysis on the same sample included in the phase 2 ondansetron study described above that considered multiple SNPs located on genes for the 5-HT_{3A} and 5-HT_{3B} subunits of the 5-HT_3 receptor. The latter analysis (Johnson et al. 2013) investigated the associations between drinking severity and 18 SNPs across *HTR3A* and *HTR3B* genes using blood samples for genetic testing and ondansetron response data from the Phase 2 trial of ondansetron (4 µg/kg, twice daily) versus placebo in 283 alcohol-dependent individuals with or without the LL/TT genotypes of the 5-HTT (described above: Johnson et al., 2011). The analysis focused on 18 SNPs selected based on their minor allele frequency (≥ 0.05), their physical distribution on the *HTR3A* and *HTR3B* genes to obtain uniform physical coverage, and results from previous genetic association studies.

This analysis identified three *HTR3A* and *HTR3B* genotypes that were significantly associated with a positive response to ondansetron in the EA sample (see Table 1). Because of population differences in allele frequencies and linkage disequilibrium structure, the study of AAs will examine ondansetron response in relation to genetic variants that overlap, but are not identical, with those being examined in the EA sample (see section 3.1 below).

Table 1: HTR3A and HTR3B genotype associations with response to ondansetron in EAs

Gene	SNP	Sample size	Outcome measure: Estimated mean difference (95% C.I.); P value		
			DDD	PHDD	PDA
HTR3A	rs1150226 (AG)	O =20; P=24	-1.81(-3.51 to -0.12); 0.036	-20.64 (-36.17 to -5.12); 0.009	19.75 (4.31 to 35.19); 0.012
	rs1176713 (GG)	O =6; P=9	-3.92 (-7.00 to -0.84) 0.013	-24.36 (-52.19 to 3.47); 0.086	18.21 (-9.27 to 45.69); 0.194
HTR3B	rs17614942 (AC)	O =17; P=19	-2.73 (-4.59 to -0.87) 0.004	-20.45 (-37.58 to -3.32); 0.019	17.97 (0.97 to 34.96); 0.038

All comparisons are between ondansetron vs. placebo groups. O – Ondansetron; P- Placebo

Use of a larger ancestry-informative SNP panel for assessment of population admixture between samples in the two treatment groups:

Allelic differences among populations are potential confounders in genetic association analyses, which may result in spurious associations. The differences in population admixture between subjects included in ondansetron and placebo groups of the Phase 2 study described above were tested using the program STRUCTURE (http://pritch.bsd.uchicago.edu/software/structure2_2.html), which revealed no statistically significant difference between the two groups. As the subjects consisted of two groups, Caucasians and Hispanics, individual genetic ancestry proportions were calculated for all 283 subjects and were used as covariates in all of the above-mentioned statistical models that tested *HTR3A*, *HTR3B* and *SLC6A4* genotype effects. Genetic ancestry proportions are increasingly being used as covariates in genetic association analyses as a strategy to limit type I error (Piwkham et al., 2011; Sucheston et al., 2011) [1-3]. In this Phase 2 trial, to prevent spurious associations resulting from population stratification, genetic ancestry proportions will be assessed using a panel of 186 ancestry informative SNPs described by Hodgkinson et al., (2008) as opposed to the 24-marker panel that we used in the phase 2 study. Genetic ancestry proportions will be calculated for each individual subject during data analysis, using the statistical software package *Structure* (Pritchard, Stephens, & Donnelly, 2000) [4] and genetic ancestry proportions for each individual subject will be used as covariates in statistical analyses.

1.5 Dose Rationale and Risk/Benefits

Dose Selection: Selection of the 0.33 mg dose is based on the results of prior Phase 2 studies (Johnson et al., 2011; Johnson et al., 2000; Sellers et al., 1994)). Johnson et al. (2011) [1-3] showed that ondansetron at a dose equivalent of 0.33 mg twice daily was well tolerated and effective in reducing alcohol consumption in subjects with targeted genotypes vs. subjects without the targeted genotypes. Based on that study, we chose a dosage of 0.33 mg twice daily of ondansetron for use in the present trial.

Each subject will receive ondansetron doses at the CNC under direct observation during the baseline visit (first dose at study Week 0) and on study Weeks 4 and 8 (first dose of the week). All other doses will be take-home. The investigator will closely monitor the subject for any adverse events or lack of tolerability. Subjects will continue twice-daily dosing at home, and will be counseled to take the study medication at approximately the same times each day.

Subjects will be asked to return all unused study medication and packaging to the study staff. Throughout the study, the staff will closely monitor the subject for dosing compliance and adverse events or lack of tolerability to the study medication. If the subject stops taking study medication for any reason and later, upon discussion with the investigator, wishes to restart the study medication, he/she will be provided with that opportunity no later than the study visit immediately following the request. Interruption in study medication will be documented on the case report form. If the subject wishes not to restart the medication, he/she will be encouraged to continue to participate in all scheduled study visits and assessments. The case report form will document the duration through which the subject took study medication.

Contraindications: The concomitant use of **apomorphine** with ondansetron is contraindicated based on reports of profound hypotension and loss of consciousness when apomorphine was administered with ondansetron. Ondansetron is also contraindicated for patients known to have hypersensitivity to the drug.

Warnings:

Hypersensitivity reactions have been reported in patients who have exhibited hypersensitivity to other selective 5-HT_3 receptor antagonists.

ECG changes including QT interval prolongation has been seen in patients receiving ondansetron. In addition, post-marketing cases of Torsade de Pointes have been reported in patients using ondansetron. Ondansetron should not be administered to patients with congenital long QT syndrome. ECG monitoring is recommended in patients with electrolyte abnormalities (e.g., hypokalemia or hypomagnesemia), congestive heart failure, bradyarrhythmias or patients taking other medicinal products that lead to QT prolongation.

High-dose, intravenous ondansetron (32 mg) has been associated with prolongation of the QTc interval. Freedman et al. (2014) reviewed the risk of cardiac arrhythmia associated with ondansetron). Over the 22 years since ondansetron has been approved, no one case of an arrhythmia following a single oral dose of ondansetron has been reported. Furthermore, the maximal QTc lengthening from a 32 mg IV dose (100 x the dose proposed in the study) of ondansetron is 17-20 msec but only 5.8 msec after an 8 mg oral dose (24 x the dose proposed in this study). Additionally, the peak serum level of oral ondansetron is only 3 to 35% of IV ondansetron. Individuals identified by Freedman et al. (2014) to be most at risk of developing an arrhythmia were treated with ondansetron IV and had either an underlying arrthymogenic condition or were receiving other pro-arrthymic medications. As a precaution, we have incorporated appropriate exclusion criteria (long QTc syndrome) even though it would require that approximately 16,000 people be screened by ECG to find one case of asymptomatic long-QT syndrome. A 12-lead ECG assessment will be performed prior to the initiation of drug therapy and at visit 5, week 5 to assess possible effects of the currently proposed chronic low dose of ondansetron (0.66 mg/day) on the QTc interval. Subjects will also be screened for

electrolyte abnormalities, which are not uncommon in this patient population and could lead to cardiac arrhythmias.

Serotonin syndrome has been reported with 5-HT_3 receptor antagonists alone. Most reports have been associated with concomitant use of serotonergic drugs (e.g., selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), monoamine oxidase inhibitors, mirtazapine, fentanyl, lithium, tramadol, and intravenous methylene blue). Some of the reported cases were fatal. Serotonin syndrome occurring with overdose of ondansetron alone has also been reported. The majority of reports of serotonin syndrome related to 5-HT_3 receptor antagonist use occurred in a post-anesthesia care unit or an infusion center. Serotonin syndrome may include the following combination of signs and symptoms: mental status changes (e.g., agitation, hallucinations, delirium, and coma), autonomic instability (e.g., tachycardia, labile blood pressure, dizziness, diaphoresis, flushing, hyperthermia), neuromuscular symptoms (e.g., tremor, rigidity, myoclonus, hyperreflexia, incoordination), seizures, with or without gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea). Patients should be monitored for the emergence of serotonin syndrome, especially with concomitant use of ondansetron and other serotonergic drugs. If symptoms of serotonin syndrome occur, discontinue ondansetron and initiate supportive treatment. Patients should be informed of the increased risk of serotonin syndrome, especially if ondansetron is used concomitantly with other serotonergic drugs.

Drug Interactions**:** Ondansetron does not appear to induce or inhibit the cytochrome P-450 drug-metabolizing enzyme system of the liver but is metabolized by this system. Theoretically, inducers or inhibitors of these enzymes may change the clearance and, hence, the half-life of ondansetron. On the basis of available data, however, no dosage adjustment is recommended for patients on these drugs. Thus, we will inform patients being treated with serotonergic antidepressants that the potential exists for an interaction with the study medication and we will monitor their progress accordingly. It should be noted, however, that the low dose of ondansetron that is being used in this study (0.66 mg/day), is unlikely to contribute to serotonin syndrome. Notably, the dosage of ondansetron that is commonly prescribed to prevent nausea and vomiting is 16 mg/day, thus the dosage used in the current study is less than 1/24 the anti-emetic dosage.

- Apomorphine**:** Based on reports of profound hypotension and loss of consciousness when apomorphine was administered with ondansetron, concomitant use of apomorphine with ondansetron is contraindicated.
- Phenytoin, Carbamazepine, and Rifampicin*:* In patients treated with potent inducers of CYP3A4 (i.e., phenytoin, carbamazepine, and rifampicin), the clearance of ondansetron was significantly increased and ondansetron blood concentrations were decreased. However, on the basis of available data, no dosage adjustment for ondansetron is recommended for patients on these drugs.
- Tramadol: Although no pharmacokinetic drug interaction between ondansetron and tramadol has been observed, data from 2 small studies indicate that ondansetron may be associated with an increase in patient controlled administration of tramadol.
- Serotonergic Drugs: Serotonin syndrome (altered mental status, autonomic instability, and neuromuscular abnormalities) has been associated with the concomitant use of 5-HT_3 receptor antagonists and other serotonergic drugs, including selective serotonin reuptake inhibitors (SSRIs; e.g. fluoxetine, sertraline, and citalopram,) and serotonin and noradrenaline reuptake inhibitors (SNRIs; e.g. venlafaxine). As indicated above, the likelihood of such an interaction is very small given the very low dosage of ondansetron being used in this study. To ensure the safety of participants, we will monitor the appearance of signs and symptoms of the serotonin syndrome at every study visit after screening, using a standardized Serotonin Syndrome Symptom Checklist. Two or more positive responses on the Serotonin Syndrome Symptom Checklist will trigger further clinical evaluation. If there is clinical suspicion of the serotonin syndrome, the study

medication will be immediately stopped and the participant told to also immediately stop taking the serotonergic concomitant medication. (See section 5.6).

Carcinogenesis, Mutagenesis, Impairment of Fertility: Ondansetron was not mutagenic in standard tests for mutagenicity.

Pregnancy: Teratogenic Effects: Pregnancy Category B. Should a woman become pregnant at any point in the study, we will immediately discontinue the treatment and refer her to an obstetrician for evaluation. We will also maintain contact with her via phone for the duration of the pregnancy to obtain self-reported safety information and, with her permission and that of her physician, will obtain information from her medical chart.

Adverse Reactions: Studies examining adverse events associated with ondansetron (at a dosage much higher than the one being used in the present study) have focused on two patient groups: individuals receiving high-dose chemotherapy and subjects being treated for post-operative nausea and vomiting. Ondansetron was well tolerated in the postoperative nausea and vomiting population (n = 1081), which is more representative of our proposed population. In this group, adverse events were similar between ondansetron and placebo (see Table 2)

Post-marketing reports: Post-marketing reports are voluntary, therefore the frequency of the following adverse events are unknown: transient ECG changes (rare and predominantly with intravenous ondansetron); flushing; hypersensitivity reactions (rare), sometimes severe (e.g., anaphylaxis/anaphylactoid reactions, angioedema, bronchospasm, shortness of breath, hypotension, laryngeal edema, stridor); allergic reactions, including laryngospasm, shock, and cardiopulmonary arrest (predominantly the injectable formulation). Also reported: liver enzyme abnormalities, hiccups, oculogyric crisis (alone, as well as with other dystonic reactions), urticaria, and transient blindness (predominantly with the intravenous formulation and resolved within a few minutes up to 48 hours).

Table 2: Adverse Events Associated with ondansetron and placebo

Adverse Event	ondansetron 16 mg	Placebo
Wound problem	152 (28%)	162 (31%)
Drowsiness/sedation	112 (20%)	122 (23%)
Headache	49 (9%)	27 (5%)
Hypoxia	49 (9%)	35 (7%)
Pyrexia	45 (8%)	34 (6%)
Dizziness	36 (7%)	34 (6%)
Gynecological disorder	36 (7%)	33 (6%)
Anxiety/agitation	33 (6%)	29 (5%)
Bradycardia	32 (6%)	30 (6%)
Shiver(s)	28 (5%)	30 (6%)
Urinary retention	28 (5%)	18 (3%)
Hypotension	27 (5%)	32 (6%)
Pruritus	27 (5%)	20 (4%)

Physical Risks: Peripheral venipuncture poses a small risk of bruising and local infection at the needle puncture site.

Minimization of the risks: This risk of bruising and local infection at the needle site will be minimized by adherence to sterile techniques and standard, simple procedures to ensure hemostasis.

Psychological Risks: Psychological risks can include distress and heightened sensitivity, which can occur as part of the behavioral treatments, clinical interviews, or completion of self-reported ratings and questionnaires. While the possibility of such events is low, we will monitor these situations and provide an appropriate level of support.
Such events will be documented fully in the case records.

Minimization of the risks: The potential for psychological harm will be reduced by adherence to FDA guidelines for Good Clinical Practice and by formal supervision and training of clinical personnel. Subjects will be allowed to take session breaks at any time, or reschedule a session (consistent with the overall study schedule). Experienced clinical personnel are available at the CNC to evaluate subjects who express distress or discomfort over study procedures. We have surveyed our subjects and found good satisfaction with the quality of care that they have received.
Overall, the development of carefully standardized and supervised SOP has allowed us to develop a high standard of care

Social and Legal Risks: Social and legal risks can include the disclosure of sensitive information about a subject to some other agency or an employer.

Minimization of the risks: The likelihood of information from our clinic being disseminated without the subject's consent to an outside source is remote. No information about a subject is disclosed without his or her written authority. Case records also are kept at a secure location in locked files. Access to these files is restricted to known personnel, who must fill out an entry form to receive any subject's records. Computer based information contains subject numbers and codes that cannot be traced directly back to the original records. The computer records are protected by passwords and encrypted files that meet the C2 security standards of the U.S. Department of Defense.

Risk of participation becoming part of the medical record: If a participant needs to be seen by a non-study physician and/or admitted to the hospital during the study, in order to provide medical treatment; it may be necessary to inform treatment providers (medical staff) of his/her participation in this study. This may include information regarding the use of alcohol in this study. This information can become part of the participant's medical records. When insurance companies review medical record information, they might see the documentation of alcohol use and/or abuse as justification for denying coverage or charging higher premiums. Also it is possible for medical records to be entered as evidence in court.

Risks associated with questionnaires: Participants will be asked questions of a sensitive nature concerning their medical, psychiatric, drug, and personal history, which some may find anxiety-provoking.

Minimization of risk: To minimize this risk, research personnel are highly trained to be sensitive and responsive. All answers will be strictly confidential. In addition, a federal Certificate of Confidentiality will be obtained pending IRB approval of the protocol.

Risk associated with pregnancy: The safety of ondansetron to an unborn child is not established. For these reasons, pregnant women will not be allowed to participate.

Minimization of risk: Women of childbearing potential must be using an acceptable form of birth control to participate in this study and have no intention of becoming pregnant.

Risk of alcohol withdrawal: Alcohol withdrawal may occur from suddenly stopping the use of alcohol after chronic or prolonged ingestion.

Minimization of risk: Presence and severity of acute alcohol withdrawal will be assessed at baseline and subjects who are experiencing significant alcohol withdrawal symptoms will be referred for treatment.

Risk of breach of confidentiality: The research team is highly experienced providing care and doing research of this nature.

Minimization of risk: All subject contact will occur in a private room. All study staff are well-trained and experienced in practices to protect confidentiality.

The risks to subjects in this study are low. The medication, ondansetron, is well tolerated, and we have experience with its use in alcohol-dependent subjects for whom no serious adverse events were reported. We will minimize the risks to subjects participating in this study by adhering carefully to the eligibility criteria and our SOP.

An alternative to participation in the study is for subjects to seek treatmet for alcohol use disorder from a treatment facility. Alternative treatment may include taking a medication (other than ondansetron) that is FDA-approved for the treatment of AUD (disulfiram, naltrexone, and acamprosate), or psychosocial treatment without any medication

2 Study Objectives

The primary study objective is to determine the efficacy of ondansetron 0.33 mg twice daily, administered orally for a period of 16 weeks in reducing heavy drinking (characterized by DDD) in carriers of specific SLC6A4, HTR3A and HTR3B “responsive” genotypes and secondary measures of drinking and quality of life among current drinking subjects. We will also compare ondansetron with placebo in subgroups of the sample based on alcohol intake. We also will test the additional secondary predictions that the differences seen in our primary specific aim will extend to secondary drinking measures and measures of quality of life. Additionally, we will use genome-wide expression studies of total RNA and miRNA- from blood samples and supported by supplemental funding-to compare the 15 most responsive subjects (whose drinking is reduced most significantly) and the 15 least responsive subjects on DDD and the 15 subjects with the greatest number of adverse events and the 15 with the fewest to identify changes that mediate ondansetron’s efficacy and adverse events profile, respectively.

AUD is a heterogeneous and chronic relapsing disorder that includes both acute (binge drinking) and chronic (frequent heavy drinking) dimensions. Perhaps because of this heterogeneity, the therapeutic effect size of the approved medicines for the treatment of AUD has been small (Johnson & Ait-Daoud, 2011). In an effort to overcome the heterogeneity of response to a particular medication, recent studies have instituted a personalized approach to therapy (Kranzler and McKay 2012). Major breakthroughs in pharmacogenetics have made it possible to identify discrete subgroups of the AUD population according to genetic profiles in

order to target the subjects who are most likely to respond to treatment with a particular agent. In addition, genetic variation contributing to the risk of alcohol dependence may be differentially associated with treatment response (Johnson et al., 2011; Kranzler et al., 2014; Oslin et al., 2003). Thus, a successful personalized pharmacologic approach should ensure that targeted subgroups achieve an optimal treatment response with high predictability. Such an approach holds the potential to identify not only robust responders to treatment but also those who might have minimal or modest adverse effects from the putative therapeutic medication. From a practical clinical standpoint, a personalized medicine approach starts with a screen to identify the subject's salient genotypes, in order to match the subject with the appropriate medication to ensure a high probability of a positive treatment outcome. This approach has the potential to exert a substantial positive impact on public health (Kranzler and McKay, 2012).

Hypothesis:

Our overarching hypothesis is that, in both AAs and EAs, ondansetron 0.33 mg twice daily will decrease the intensity of drinking (i.e., the number of DDD) more than placebo. We further hypothesize that the effect will be greater in specific genotype groups in the two populations and we will balance the randomization on this genetic variation separately by population group to explore this prediction.

Other exploratory aims will be examined in separate strata across both study sites, with treatment assignment at randomization balanced based on the genotypes that are relevant to each population: to test the hypothesis that alcohol-dependent individuals with 5-HTTLPR L_AL_A genotype in *SLC6A4* (which encodes the serotonin transporter protein). We also will test the secondary predictions that the differences in our primary specific aims will extend to secondary drinking measures and measures of quality of life.

Finally, we will use

genome-wide expression studies of total RNA and miRNA- from blood samples and supported by supplemental funding- to compare the most responsive subjects (whose drinking is reduced most significantly) and 15 least responsive subjects on DDD as well as the 15 subjects with the greatest number of adverse events and the 15 with the fewest to identify changes that mediate ondansetron's efficacy and adverse events profile, respectively.

3 Study Design

3.1 General Design

This is a, randomized, double-blind, placebo-controlled, parallel-group, phase 2 clinical trial to explore the safety and efficacy of ondansetron to reduce drinking intensity in AA and EA adult subjects with AUD who commonly engage in heavy drinking . We will use selected genotype polymorphisms of the serotonin transporter and 5-HT_{3B} receptor genes to examine ondansetron effects in subgroups based upon genotype. Analyses based on genotype will be conducted separately in EA and AA because different, but overlapping, genetic variants are relevant as potential moderators of ondansetron response in the two populations. The UPENN site is conducting a parallel study with both EAs and AAs.

Probability of group assignment and potential for subjects to be randomized to placebo group:

<u>European Americans</u>

Participants with EA race will be selected based on absence/presence of any one of the combination of the following genotypes and will have equal probability (i.e., 50-50 chance) of being assigned to receive ondansetron (active medication) or placebo.

Subjects will be balanced based on their *SLC6A4* genotypes, • 5-HTTLPR:LL together with rs25531:AA (L_AL_A genotype) in SLC6A4 gene;

- SLC6A4-rs1042173: TT;
- HTR3A-rs1150226: AG;
- HTR3A-rs1176713: GG;
- HTR3B-rs17614942: AC

African Americans

Participants with AA will be selected based on having a CC or TC genotype at rs176744, with each participant having an equal probability (i.e., 50-50 chance) of being assigned to receive ondansetron (active medication) or placebo.

Participants will be balanced based on their *SLC6A4* genotypes:

- 5-HTTLPR-L_AL_A vs. non- L_AL_A genotypes
- rs1042173*TT vs. TG/GG genotypes.

….

Eligible subjects will be stratified into two groups (Genotype responsive vs. non-responsive). Genotype responsive group will include the carriers of the above mentioned genotypes and the non-responsive group will include those who do not carry any of the above genotypes.

Genotypes will be determined within one week of the Screen Visit. Primary Study Endpoints

Drinking variables will be derived from the data collected by the Timeline Follow-back Interview (TLFB). Baseline for the drinking variables is defined as the 90 days preceding the baseline visit. One month is defined as 4 weeks (28 days).

Primary Efficacy Variable: Change from baseline in the number of drinks per drinking day (DDD) during the last 8 weeks of the 16 week treatment period.

3.2 Secondary Study Endpoints

Key Secondary Variables

- Responder analysis: Number of subjects with no HDDs during the last 8 weeks of the 16-week treatment period.
- Change from baseline in the total alcohol consumption, defined as mean daily alcohol consumption in standard drinks/day over the last 4 weeks of the 16-week treatment period.

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Change from baseline in the percentage of heavy drinking days (PHDDs) defined as a day on which men drink >5
drinks and women drink >4 drinks.

3.3 Primary Safety Endpoints

Safety evaluations, including laboratory assessments, physical examination, vital signs, 12-lead ECG and assessment of alcohol withdrawal, depression and suicidal risk, and querying for adverse events will be performed to identify safety issues in the study.

Hematology, clinical chemistry, urinalysis, urine drug screen, medical history, psychiatric history, and physical examination will be performed at baseline and at varying intervals during the 16-week treatment, as described in detail in Table 3 below.

Body weight and vital signs (including temperature, systolic and diastolic blood pressure, heart rate and respiratory rate) will be measured at screening/baseline and at every study visit.

The Clinical Institute Withdrawal Assessment for Alcohol scale (CIWA-Ar) is a reliable and well-validated, 10-item scale to assess the severity of alcohol withdrawal symptoms. Alcohol withdrawal symptoms at the screening visit constitute a screen failure. Any post-screening physical finding from the CIWA-Ar that is considered to be clinically significant will be reported as an adverse event.

The 21-item Beck Depression Inventory (BDI) will be used to measure depressive symptoms. Questions focus on topics such as mood, suicidal thoughts, sleep habits, and eating behaviors. The BDI will be administered at every study visit. At screening/baseline, subjects with severe, untreated depression or who endorse suicidal intent will be excluded from the study and referred for treatment or immediate intervention. At each post-baseline visit, the staff will review the findings on the BDI to ensure that the subject's safety is not compromised by changes in depression symptoms.

Careful screening using the MINI International Neuropsychiatric Interview 6.0 (MINI) Suicide Risk Assessment Scale (module B) will serve to exclude individuals with significant suicidal risk. Ongoing monitoring using that instrument throughout the study will serve to reduce the risk of intentional overdose or other suicidal behavior. The MINI B will also be used to assess suicide risk at each study visit, and if rated equal to or greater than 9 (i.e., clinically significant suicidal ideation), a study physician will be consulted to decide the appropriate clinical management.

Table 3: Schedule of Study Procedures

Assessments																			
Study Visit	V0-1 (Screen)	V0-2 (Baseline)	V1	V2	V3	V4	V5	V6		V7 (Midpoint)		V8		V9				V10(endpoint)	V11
Study Week	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**20**
BrAC[a]	X	X	X	X	X	X	X	X		X		X		X				X	X
Weight, vital signs, height[b]	X	X	X	X	X	X	X	X		X		X		X				X	X
TLFB[c]	X	X	X	X	X	X	X	X		X		X		X				X	X
SCREENIING																			
Informed Consent	X																		
SORT	X																		

Race/Ethnicity Questionnaire	X																	
Medical and Psychiatric History, Demographics	X																	
SCID-IV, AUDIT[d]	X																	
SSADDA Nicotine Dependence Module	X																	
AUD criteria and clinical status (DSM-5)	X																X	
Names and contact information for two locators		X																
LABORATORY TESTS																		
Blood sample for genotyping	X																	
Blood sample for hematology & clin. chem.	X																X	
Blood sample for %dCDT and GGTP	X									X							X	
Blood sample for mRNA and miRNA expression studies	X					X				X				X			X	X
Urinalysis	X																X	
Urine drug screen	X													X			X	
Urine pregnancy test	X	X				X				X				X			X	
Urine specimen for riboflavin		X	X	X	X	X	X	X		X		X		X			X	
OTHER ASSESSMENTS																		
Other Drug Use questionnaire		X	X	X	X	X	X	X		X		X		X			X	
SF-12, SIP		X												X			X	
OCDS, PSQI		X	X	X	X	X	X	X		X		X		X			X	
ADS		X								X							X	
STUDY VISIT	**V0-1 (Screen Part-1)**	**V0-2 (baseline)**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**		**V7**		**V8**		**V9**			**V10**	**V11**
STUDY WEEK																		
NON-LABORATORY CLINICAL MONITORING																		
Physical exam	X																X	
ECG		X					X											
CIWA-Ar	X	X	X	X	X	X	X	X		X		X		X			X	
BDI		X	X	X	X	X	X	X		X		X		X			X	
MINI Suicide Risk Assessment Scale	X	X	X	X	X	X	X	X		X		X		X			X	
Clinical Global Impression Scales (CGI) (CGI-I, CGI-S)		X	X	X	X	X	X	X		X		X		X			X	

BBCET		X	X	X	X	X	X	X		X		X		X				X	
SSS Checklist[h]		X	X	X	X	X	X	X		X		X		X				X	
STUDY MEDICATION																			
Administration of Study Drug (witnessed ingestion)		X				X				X									
Dispensing of study drug		X	X	X	X	X	X	X		X		X		X					
Compliance and drug accountability			X	X	X	X	X	X		X		X		X				X	
Adverse events[e]			X	X	X	X	X	X		X		X		X				X	X[i]
, Concomitant medications and treatments		X	X	X	X	X	X	X		X		X		X				X	X[g]

a. BrAC will be the first procedure at all visits;
b. Height measured at screen part-1 visit only;
c. At screen part-1 visit, subjects will complete the TLFB for the preceding 90 days. At subsequent visits, the TLFB will span the time since the prior visit;
d. AUDIT (at phone screen)
e. Subjects will be monitored for the occurrence of adverse events from the date the informed consent is signed;
f. Final visit can be a telephone call unless an SAE has occurred since the last visit. Safety follow-up should include subjects who withdrew their consent but the safety follow-up data must only be included in the medical records and not in the CRF.
g. Adverse events only
h. Only for participants taking a serotonergic concomitant medication

Abbreviations in the table: ADS=Alcohol Dependence Scale; AUD=Alcohol Use Disorder; AUDIT=Alcohol Use Disorders Identification Test; BBCET=Brief Behavioral Compliance Enhancement Treatment; BDI=Beck Depression Inventory; CGI-I & CGI-S=Clinical Global Impression Scales (Improvement & Severity); CIWA-Ar=Clinical Institute Withdrawal Assessment for Alcohol-revised; GGTP=gamma-glutamyltranspeptidase; dCDT=disialotransferrin; MINI=Mini International Neuropsychiatric Interview; PSQI=Pittsburgh Sleep Quality Index; SCID=Structured Clinical Interview for DSM-IV; SF-12=Short Form Health Survey; SIP=Short Index of Problems; SORT= Slosson Oral Reading Test; SSS Checklist = Serotonin Syndrome Symptom Checklist

4 Subject Selection and Withdrawal

Men and women, 18 to 70 years old, will b recruited: 183 AA and 183 EA. EAs will be self-identified as White, non-Hispanic or Hispanic individuals. Approximately 30% of the total cohort will be women. Minorities and women will, therefore, be well represented in this trial. Children between the ages of 18 years and 21 years will be included in this study. This is in accordance with NIH guidelines for the inclusion of children in clinical studies, if appropriate. The inclusion of children enhances the generalizability of the results of clinical studies to the general population.

The ethnic group commonly known as “Hispanic” or “Latino” is an admixed population that primarily resulted from two-way admixture between Native American and European populations or three-way admixture among Native American, European, and West African populations. In admixed individuals with a disease, chromosomal segments harboring susceptibility variants will show an excess of ancestry from the parental population in which the risk alleles are more frequent (Mao et al., 2007), leading to genetic subgroups within a population. The existence of genetic subgroups or substructure in a population may lead to spurious associations if the subgroups are not equally represented in cases and controls (Lai et al., 2009). Balancing for subgroups in a genetic association study examining a common disorder such as AUD will require a large number of subjects to permit an analysis of the subtle effects of multiple polymorphisms with sufficient statistical power. For this reason, the current study will

be limited to subjects of predominantly (>75%) European and African ancestry. This will include Hispanic/Latino individuals who self-report being of African or European ancestry.

Number of subjects (at each site) needed to complete the protocol: 128 subjects of African-American ancestry and 128 subjects of European ancestry are needed to complete the protocol. Also, each site will aim to have a total of 128 completing subjects (from either race).

Expected rate of screen failure/ dropouts/withdrawals from all sites: Allowing for a 30% drop-out rate (during the course of the study, which is about 4 months), as seen in previous studies conducted by the PI's research team, we will need to randomize about 183 subjects at each site in order to have 128 subjects complete the protocol.

Inclusion Criteria

- Men and women who have given written informed consentAged 18 to 70.
- The subject has a breath alcohol concentration (BrAC) = 0.00% at the screening visit
- Self-reported African-American or European ancestry (mixed ancestry individuals will not be included in this study)
- The subject has a breath alcohol concentration (BrAC) = 0.00% at the screening visit
- Diagnosis of alcohol use disorder (AUD) using Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) criteria
- Able to provide TLFB alcohol consumption information for the 90-day period prior to the Screen Part-1 Visit.
- During the 4 weeks preceding the Screening Visit, the subject reports:
    - ≥6 Heavy Drinking Days (HDDs) – defined as a day with alcohol consumption of ≥ 5 standard drinks (i.e., 12 g of ethanol) for men, and ≥ 4 standard drinks for women
    - ≤14 consecutive abstinent days
    - Total alcohol consumption of an average of ≥21 standard drinks/week for men and ≥14 standard drinks/week for women in past 30 days and have met these criteria during the 7 days prior to randomization
- An expressed wish to reduce or stop drinking
- Willingness to participate in a research study with behavioral treatment and medicinal treatments or placebo study medication for Alcohol Use Disorder
- Stable residence in the 28 days prior to the Baseline Visit and no plans to move in the next 9 months. A stable residence is a domicile in which an individual can operate as if it were his or her own homestead and does not include shelters or halfway houses.
- Provides contact information for 1 or 2 "locators" who can be used to contact the subject
- Able to read and understand English and accurately complete the rating scales and questionnaires, follow instructions, and make use of the behavioral treatments. This will be assessed with the Slosson Oral Reading Test-Revised, on which the subject must demonstrate at least a 6th grade reading level.
- If the subject is a woman of child-bearing age, she must:
    - Agree not to try to become pregnant during the study, and use adequate contraception (defined as oral/ systemic contraception, such as Norplant, Depo-Provera, or birth control pills or patches, an intrauterine device, a combination method such as a diaphragm or male condom combined with spermicide, jellies or foam, withdrawal, a sponge, the rhythm method, or a cervical cap) or

- Be postmenopausal, (i.e., have had her last natural menstruation at least 24 months prior to baseline) or
- Have had a hysterectomy or been surgically sterilized prior to the Baseline Visit, or
- Plan not to be sexually active vaginally with men during the entire duration of the trial.
- Weight >110 Kg (242 lb)

Exclusion Criteria

A subject presenting with any of the following criteria will be excluded from the study:

- The subject has fewer than 6 heavy drinking days (HDD) (defined as ≥5 standard drinks for men and ≥4 or greater standard drinks for women) in the 4 weeks preceding the Screening Visit.
- The subject has greater than 14 consecutive abstinent days in the 4 weeks preceding the Baseline Visit.
- The subject has a Clinical Institute Withdrawal Assessment for Alcohol Scale (CIWA-Ar), Revised, score ≥10 at Baseline visit.
- The subject has a current diagnosis of any of the following psychiatric disorders: schizophrenia, bipolar disorder, other psychotic disorder, severe major depressive disorder, post-traumatic stress disorder, panic disorder, eating disorder, or substance use disorder (except alcohol, tobacco, or cannabis).
- Current or recent (within 4 weeks prior to Baseline Visit) treatment with antipsychotics or any medications likely to interact with ondansetron to produce adverse effect, as judged by a study physician .
- Treatment with any investigational medicinal product within 30 days or 5 half-lives (whichever is longer) prior to the Randomization
- Currently participating or has recently (4 weeks prior to the Randomization) participated in a treatment program for alcohol use disorders.
- MINI 6.0 Suicide Risk Assessment (module B) will be used to assess subjects' risk of suicide. Subjects who receive a score of ≥9 on this assessment will be evaluated by the PI or designee to determine their eligibility. Subjects who are deemed by the PI or designee to be at risk of suicide will be excluded.
- Clinically significant, unstable physical illness (e.g., hematologic disorders, hepatic or renal insufficiency, or a cardiovascular, pulmonary, gastrointestinal, endocrine, neurological, infectious, neoplastic, or metabolic disturbance), as judged by the PI or designee to be exclusionary
- Clinically significant abnormal vital signs, as judged by the PI or designee
- Clinically significant abnormal 12-lead ECG at the Screen Visit, clinically significant cardiovascular disease requiring regular or intensive clinical monitoring, or a current or past history of clinically significant QT prolongation, including: QTcF > 450 ms (average of 3 12-lead measurements)

- Serum potassium, magnesium or calcium levels outside the central laboratory's reference range that are deemed clinically significant by the PI or designee.

Taking medication (within the last 7 days prior to the Baseline Visit) that has the potential to clinically significantly prolong the QT interval, as judged by a study physician. or may require such medications during the course of the study (see Table below for a list of medications). For patients taking these medications, a study physician will evaluate the potential for ondansetron to interact with the medication to produce a clinically significant risk for the participant.

- Clinically unstable cardiac disease, including unstable atrial fibrillation, symptomatic bradycardia, unstable congestive heart failure, active myocardial ischemia or indwelling cardiac pacemaker
- Complete left bundle branch block
- History of Long QT Syndrome or a first-degree biological family member with this condition
  - Evidence of hepatic failure and/or ascites, prolonged prothrombin time (International Normalized Ratio [INR] ≥1.7), bilirubin >10% above the upper limit of the central lab's normal range and/or esophageal variceal disease
  - Active hepatitis and/or serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) or lactate dehydrogenase (LDH) > 3x the upper limit of normal
  - Treatment, either current or within 28 days prior to the Randomization, with any medications having a potential effect on alcohol consumption and related behaviors. These include opioid antagonists (e.g., naltrexone, Vivitrol®, Selincro®), glutamate antagonists (e.g., acamprosate), anticonvulsants (e.g., topiramate, gabapentin), dopamine antagonists (e.g., haloperidol), and disulfiram (Antabuse®)
  - At the Screen visit, the subject's urine contains opiates, cocaine, methamphetamines, benzodiazepines, or opiates that cannot be explained by appropriate use of a prescribed medication.
  - History of severe or life-threatening adverse reactions to ondansetron
  - Female subjects of childbearing potential who have a positive pregnancy test at Baseline Visit or are pregnant, breast feeding, not adhering to an acceptable form of contraception at screening or any time during the study, or unwilling to maintain an acceptable form of contraception throughout the study
  - Prior to randomization, the subject is compelled to participate in an alcohol treatment program to maintain his/her liberty
  - As of Screen Visit, the subject is sharing a household with a subject randomized to any investigational trial of ondansetron
  - Any other condition or therapy that, in the investigator's opinion, may pose a risk to the subject, prevent the subject from completing the required study procedures or interfere with the study objectives
  - Less than 75% European ancestry proportions or African-American ancestry proportions. If ancestry is determined by a 24-marker ancestry panel, the participant will not start treatment until after the results are known.

Table 4

Some drugs associated with QTc prolongation

Antibiotics	**Anaesthetics**	**Antipsychotics**
azithromycin	halothane	risperidone
clarithromycin		fluphenazine
erythromycin	**Antiarrhythmics**	haloperidol
roxithromycin	disopyramide	clozapine
metronidazole (with alcohol)	procainamide	thioridiazine
moxifloxan	quinidine	ziprasidone
		pimozide
Antifungals		droperidol

fluconazole
(in cirrhosis)
ketoconazole

Antivirals
nelfinavir

Antimalarials
chloroquine
mefloquine

amiodarone
sotalol

Antidepressants
amitriptyline
clomipramine
imipramine
dothiepin
doxepin

Antihistamines
terfenadine*
astemizole*

Other
probucol
cisapride

4.1 Subject Recruitment and Screening

Subjects will be recruited at research centers at the Clinical Neurobehavioral Center (CNC) at UMB. Advertisements for the study will state clearly that there will be no cost to the subject for participation. Only recruitment materials and methods approved by the UMB IRB will be utilized. We recognize that our recruitment efforts may yield individuals in need of treatment who do not qualify for the present study. We will try to enroll these individuals in other ongoing studies at our sites. Should subjects fail to qualify or decline to participate in other ongoing treatment studies at our research centers, we will assist with a referral to another treatment facility.

Subjects will be recruited through local advertisements, possibly including posters, flyers, newspaper, radio, television, electronic communications (including email and social media), Craigslist, advertisements on mass transit, referrals from local treatment programs and centers, and commercial sites such as such as gambling casinos, race tracks (horse, auto), hunting and fishing supply stores, auto supply stores, and stores selling alcoholic beverages.

University health systems, community agencies, and local college campuses that offer services and means of posting and distributing recruitment materials in the community settings with public posting areas or providing community access to materials (such as hospitals, town halls, public libraries, YMCAs, health fairs, etc.) We will obtain permission at selected locations before distributing or posting the approved recruitment materials (ensuring compliance with other institution guidelines, including seeking IRB approval as needed). We will also screen patient databases as permitted by the institution and approved by the IRB. We will search the University of Maryland Medical Center and FPI EPIC medical record databases to identify potential subjects based on demographics, medical diagnosis codes related to AUD and exclusionary comorbid diagnoses, and residence within commuting distance of CNC. We will contact the individuals with IRB-approved outreach letters from the study PI. About a week after sending the letter, we will make a follow-up phone call to these individuals if no response has been received.

Subjects who express interest will be invited to participate in a telephone screening interview. Prior to initiating screening, we will describe the nature of the study to the subject, including the number of visits to the center, subject protections, and confidentiality. If the caller remains interested, we will obtain oral consent for the phone screening. Screening questions cover general health, psychiatric history and alcohol consumption (AUDIT, see section 6.7 below). If we determine the applicant to be eligible based on this screening, s/he will be asked whether they wish to participate. If so, s/he will be scheduled for an initial in-person appointment, at which informed consent will be obtained.

We will request a partial waiver of HIPAA Authorization from the IRB to allow for preliminary telephone screening for calls initiated or invited (e.g., following our having contacted them by letter) by potential subjects. Individuals deemed eligible for in-person screening will

sign a HIPAA Authorization and study consent form at their first visit. These procedures have been approved by the Institutional Review Boards of both UMB and UPenn to ensure compliance with HIPAA requirements. The telephone screening interview will include: a) demographic data; b) medical status; c) a brief psychiatric history; d) a brief TLFB for alcohol consumption; e) drug use history and e) questions to assess motivation to engage in treatment. Prospective participants will contact the CNC by telephone. On the telephone, recruiting staff will provide information about the trial and will provide the caller with the eligibility criteria. Recruitment staff will follow an IRB approved telephone script. If the caller remains interested in participation, he or she will be informed about a time to come to the CNC for medical screening. Our intake interviewers have been trained specifically in these tasks. This enhances data quality and increases the likelihood of suitable subjects being invited for intake. Collection of these data, which are identical to some of the measures in the proposed study, will allow for comparison of the target and study populations. Additionally, it will improve the efficiency of the study and reduce the likelihood of unsuitable subjects having to undergo a full screening before being excluded. The telephone screening interviews will be conducted only by trained study staff. Once subjects pass this telephone screen, we will schedule an intake examination within 14 days.

4.2 Early Withdrawal of Subjects

4.2.1 When and How to Withdraw Subjects

It is recognized that subjects are free to withdraw from the study at any time. However, if a subject wishes to withdraw from the study before completing all study visits during the treatment phase, the study staff will ask him or her to schedule an early termination visit. The early termination visit will include all Week 16 procedures and assessments. Following an early termination, an effort will be made to interview the subject at the previously scheduled study endpoint, either in person or by telephone. This will make it possible to record drinking history during the period between early termination and the scheduled endpoint.

The following efforts will be made to contact subjects who discontinue treatment prior to completing the 16-week treatment period: 2 telephone calls by the research coordinator or technician resulting in contact (either with the subject or by leaving a message on voicemail or a person answering the subject's telephone), followed by 2 telephone calls by a study physician, followed by calls to the 2 locators nominated by the subject, followed by a letter. Only if all of these efforts are unsuccessful in obtaining follow-up information (either during an in-person visit or a telephone interview), will the subject be considered to be lost to follow-up.

An investigator may discontinue medication and withdraw the subject from the study if he or she deems it clinically appropriate or for any of the following reasons: 1) significant side effects from the medication, 2) serious or unexpected adverse events, 3) inability to comply with the study protocol, 4) protocol violation, or 5) serious intercurrent illness. A subject may withdraw from the study anytime he or she wishes. In the event that a subject is discontinued from receiving the research medication but may be able to continue with the psychosocial intervention, the subject will be allowed to continue the psychosocial intervention visits with the approval of the investigator.

At week 16, subjects that request ongoing care will be given a referral to an alcohol treatment facility or may be given a list of alcohol treatment facilities to contact.

4.2.2 Data Collection and Follow-up for Withdrawn Subjects

Any subject who discontinues treatment prematurely, regardless of the reason, will be requested to return for a final visit to do the necessary procedures and obtain data for end-of-study/early-termination and follow-up visits. Following an early termination, an effort will be

made to interview the subject at the previously scheduled study endpoint, either in person or by telephone. This will make it possible to record drinking history during the period between early termination and the scheduled endpoint.

5 Study Drug

5.1 Description

The only investigational agent to be used in this study is ondansetron, a specific serotonin-3 (5-HT_3) antagonist that is FDA approved to treat chemotherapy-induced nausea and vomiting, radiation-induced nausea and vomiting, and postoperative nausea and vomiting. In the present study, subjects will be given ondansetron at a dosage of 0.33 mg or matching placebo capsules by mouth twice daily.

The active ingredient in ondansetron tablets is ondansetron hydrochloride (HCl) as the dihydrate, the racemic form of ondansetron. Chemically it is (±) 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one, monohydrochloride, dihydrate. Ondansetron's empirical formula is C18H19N3O•HCl•2H2O, representing a molecular weight of 365.9.

5.2 Treatment Regimen

Ondansetron 0.33 mg or matching placebo by mouth twice daily for 16 weeks.

5.3 Method for Assigning Subjects to Treatment Groups

Randomization: Subjects within each of the 2 above-mentioned genotype groups will be randomly assigned to receive ondansetron or placebo. The clinical coordinator will collate intake data essential to assign subjects to a treatment group, including genotype information, which will be used to complete a **Subject Randomization Form.** The **Randomization List Form** records the subject's ID number and UMB Investigational Drug Service (IDS) randomization to ondansetron or placebo. The UMB pharmacist is responsible to keep the treatment assignments recorded on the Randomization List Forms confidential and completely blinded from the staff involved in the trial, except as specified under the emergency conditions for which both sites have written **Standard Operating Procedures (SOPs).** Upon receipt of the Subject Randomization Form for a particular participant, the pharmacist shall communicate to the research staff which of the medication kits provided to the site is to be used for that subject. All clinical staff members directly involved with the assessment of subjects are always blind to the medication condition. These procedures have been designed to avoid delay. An IDS staff person, who is not otherwise involved in the study, will perform the randomization and assignment of eligible subjects into treatment groups. All participants and other investigators will be blinded.

Only subjects who meet all inclusion criteria and no exclusion criteria will be randomized to treatment. One hundred eighty-three subjects with a DSM-5 AUD will be randomized into two medication groups (ondansetron or placebo; N=~91/group). Randomization will be stratified by site, race, gender and genotype. There will be 8 genotype strata (based on all combinations of 3 loci or SNP's) for AAs and 2 genotype strata ("responsive" vs. "non-responsive" genotype combinations) for EAs, based on the race-specific genotypes defined in section 3.1 above). Treatment group assignment will be assigned using a block randomization procedure that balances the treatment groups within the above defined strata.

The IDS at UMB will implement the randomization for both participating sites. The clinical coordinator will collate intake data essential for assignment to a treatment group, including genotype information that will be used to complete a Subject Randomization Form. A study physician or nurse practitioner must sign the Subject Randomization Form. This signature

functions as a prescription for the UMB IDS to distribute medication to the participating sites at both universities. A copy of the form is kept in the Case Report Form (CRF), and the original is provided to the pharmacist at UMB. The UMB pharmacist will then complete all identifying information required on the Subject Dose Record Form. The Subject Dose Record Form records the subject ID number. The pharmacist is responsible to keep the dose assignments recorded on the Subject Dose Record Form confidential and completely blinded from the local investigative research staff, except as specified under the emergency conditions for which all the sites have written Standard Operating Procedures (SOPs). Upon receipt of the faxed Subject Randomization Form for a particular participant (which will be faxed to the UMB IDS Monday through Friday, from 8 AM to 4 PM), the UMB pharmacist will fax back to the site the identity of the medication kit to be dispensed to the subject. Medication starter kits for the first three weeks of treatment will be shipped in a batch to UPenn from the UMB IDS. Once the subject has been randomized, the UMB IDS will provide medication for subsequent weeks of treatment in batches, as described below. The UMB IDS will track the number of starter kits dispensed at UPenn and will replenish them to ensure that medication is available for ongoing enrollment. All clinical staff members directly involved with the assessment of subjects will always be blind to the medication condition. These procedures have been designed to avoid delay.

5.4 Preparation and Administration of Study Drug

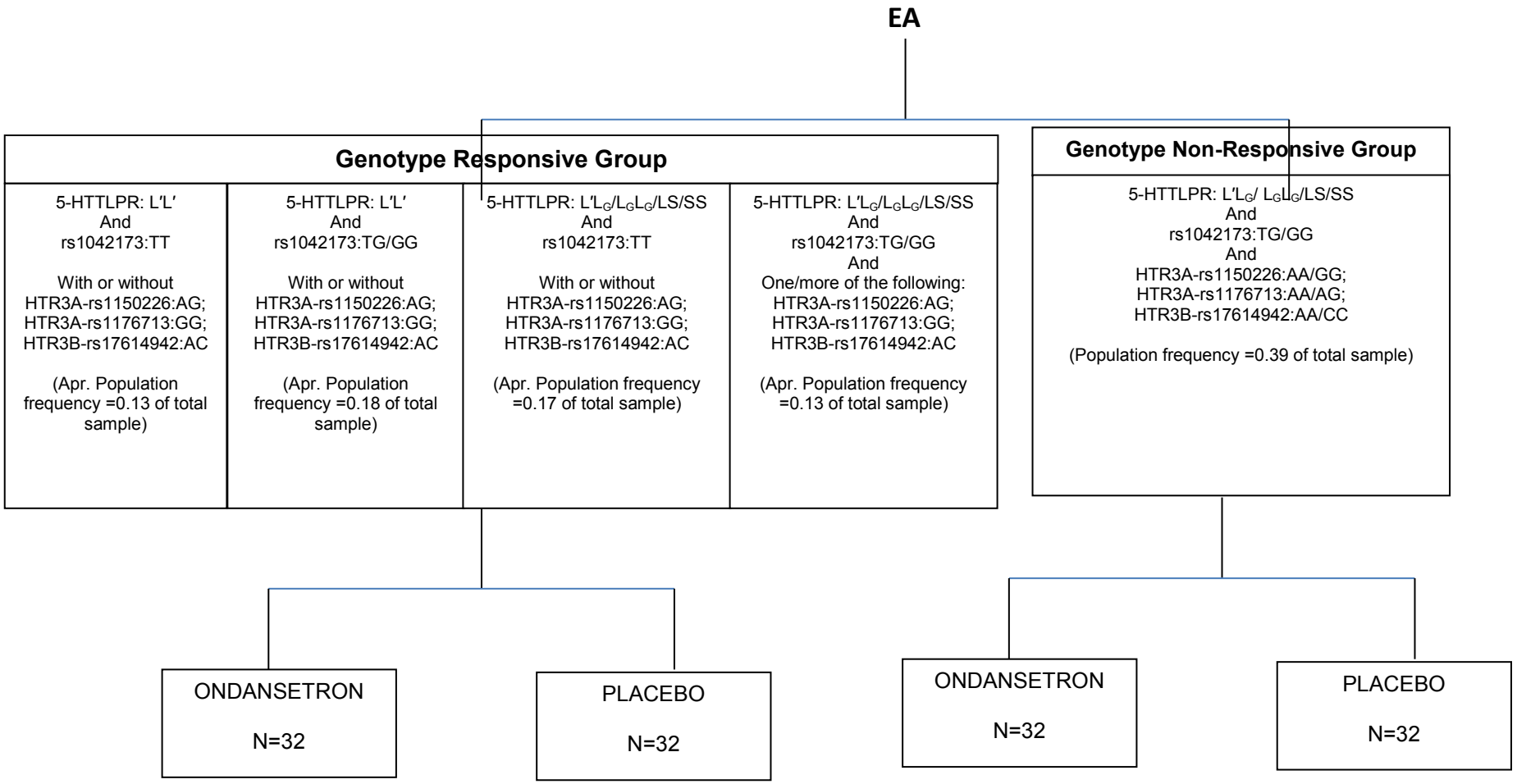
Ondansetron will be purchased commercially and formulated by the UMB School of Pharmacy Good Manufacturing Practices facility in opaque capsules. Placebo capsules will be formulated to match the active medication, so that inspection of the capsules will not allow them to be differentiated. The UMB IDS will be responsible to blind study medication according to the protocol outlined by the study statistician.

5.5 Subject Compliance Monitoring

Compliance with ondansetron will be assessed using the methods below:

- Pill Count: An account of dispensed and returned study drug will be kept at the study center for each subject. Subject reports of discrepancies (e.g., lost or discarded capsules) will be documented in the case report form (CRF).
- Presence of urinary riboflavin (an internal tracer for assessing medication compliance): Both the active and placebo drug will be packaged with the internal tracer, 12.5 mg of crushed riboflavin tablets. At screening, a research team member will query subjects as to whether they are taking any vitamins that contain riboflavin. If so, the subject will be asked to provide the name of the vitamin, and if the daily dose of riboflavin taken exceeds 2 mg, the subject will be asked to replace the formulation with a low-riboflavin formulation. Urine samples will be collected at each visit following the initiation of medication to measure riboflavin amounts in urine. Percentage of optical density of riboflavin in samples will be compared to that of a control sample containing a set amount of riboflavin to objectively determine medication compliance. Optical densities will be measured using a urinary fluorescence assay to be conducted at UMB using frozen urine samples sent from both sites.
- Dosing Diary: The clinician will use a calendar-based TLFB-style technique to elicit this information, which increases precision for the exact times of medication ingestion.
- BBCET: Brief Behavioral Compliance Enhancement Treatment (BBCET). Subjects in both medication groups will receive BBCET (described below). BBCET emphasizes maintaining compliance and avoiding early discontinuation of the medication. Adverse events are monitored and protocol-permitted remedies or over-the-counter medications are provided if necessary to deal with them.

Figure 1: Stratification and Randomization Scheme:


EA
Genotype Responsive Group
5-HTTLPR: L′L′
And
rs1042173:TT
With or without
HTR3A-rs1150226:AG;
HTR3A-rs1176713:GG;
HTR3B-rs17614942:AC
(Apr. Population frequency =0.13 of total sample)
5-HTTLPR: L′L′
And
rs1042173:TG/GG
With or without
HTR3A-rs1150226:AG;
HTR3A-rs1176713:GG;
HTR3B-rs17614942:AC
(Apr. Population frequency =0.18 of total sample)
5-HTTLPR: L′LG/LGLG/LS/SS
And
rs1042173:TT
With or without
HTR3A-rs1150226:AG;
HTR3A-rs1176713:GG;
HTR3B-rs17614942:AC
(Apr. Population frequency =0.17 of total sample)
5-HTTLPR: L′LG/LGLG/LS/SS
And
rs1042173:TG/GG
And
One/more of the following:
HTR3A-rs1150226:AG;
HTR3A-rs1176713:GG;
HTR3B-rs17614942:AC
(Apr. Population frequency =0.13 of total sample)
Genotype Non-Responsive Group
5-HTTLPR: L′LG/ LGLG/LS/SS
And
rs1042173:TG/GG
And
HTR3A-rs1150226:AA/GG;
HTR3A-rs1176713:AA/AG;
HTR3B-rs17614942:AA/CC
(Population frequency =0.39 of total sample)
ONDANSETRON
N=32
PLACEBO
N=32
ONDANSETRON
N=32
PLACEBO
N=32

5.6 Prior and Concomitant Therapy

Subjects who enter this study must not be on any medication that may affect alcohol consumption. We also will avoid the prescription of medications with potentially clinically significant interactions with ondansetron. It is, however, recognized that concomitant medication may be prescribed or self-administered to treat minor physical ailments during the study. Such appropriate medications will be allowed and documented in the concurrent medications section, and the physical ailment recorded in the adverse events section, of the subject's study book. A list of allowable concomitant medications will be compiled and placed in the appendix section of each case study book for easy reference (see the table 5 below).

Table 5: Allowed and disallowed concomitant medications

Drug Class	Episodic Use	Chronic Use
Analgesics	Y[i]	N
Anorexics	N	N
Antacids	Y	Y
Anti-anginal drugs	N	N
Anti-anxiety/alcohol withdrawal agents[ii]	N	N
Anti-arrhythmics	N	N
Anti-asthma agents	Y	Y
Antibiotics	Y	Y
Anticoagulants[iii]	N	N
Anticonvulsants	N	N
Anti-dependence drugs (e.g., bupropion or naltrexone)	N	N
Anti-diarrheal agents	Y	N
Antihistamines	Y	Y
Antihypertensives	N	Y
Anti-psychotics	N	N
Cough/cold preparations [iv]	Y	N
Diabetes medications (oral hypoglycemics, insulin)	N	Y
Dietary supplements[v]	N	N
Digitalis preparations	N	N
Hormones/steroids[vi]	N	Y
Laxatives	Y	N
Nicotine replacement therapy	N	N
Non-steroidal anti-inflammatory agents [vii]	Y	Y
Tricyclic Antidepressants	N	N

Y=allowed, N=not allowed

Definitions

Chronic use: 4 days per week or more, for a month or more. Additionally, the medication must have been started and stabilized for at least 2 weeks prior to study enrollment. Periodic or episodic use: 3 days per week or less, or more frequent use for less than a month's duration.

Key

[i] Episodic use of Opiate-containing analgesics will be allowed if there is a clear indication and the time of administration is noted relative to the timing of the urine drug screen.

[ii] Benzodiazepine use will not be allowed

[iii] Aspirin up to 325 mg/day for cardiovascular prophylaxis will be allowed.

[iv] Episodic use of opiate-containing formulations will be allowed

[v] Riboflavin is not allowed. We will allow multi-vitamins that contain less than 2 mg of riboflavin.
[vi] Topical steroid preparations for episodic use will be allowed.
[vii] Chronic NSAID use is not allowed for participants with a history of gastritis or ulcers.

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5.7 Packaging

The UMB School of Pharmacy Good Manufacturing Practice facility will package bulk drug on-site. The bottle will contain an extra two days of medication to ensure that subjects have adequate medication to continue the prescribed regimen if a visit needs to be rescheduled. Half of the participants will receive an inactive placebo. Placebo will be opaque, gelatin capsules of the same size, color, and shape packaged with cornstarch. All capsules (active and placebo) will be packaged with 12.5 mg of riboflavin for detection of urinary riboflavin to measure medication adherence.

5.8 Blinding of Study Drug

All subjects and research staff will be blinded as to whether the subject is in the ondansetron or placebo group until the decision to break the study blind is determined at the end of the study (after study database lock). Codes linking randomization number for each subject to actual treatment will be secured in a sealed, opaque envelope and maintained in a locked drawer in the IDS. Research subjects will be given the emergency contact number for the study during the consenting process. See section 8.4 (Unblinding Procedures) for a description of the process for unblinding a study subject.

5.9 Receiving, Storage, Dispensing, and Return

5.9.1 Receipt of Drug Supplies:

There is no industry sponsor for this study. The UMB School of will prepare the study medication using commercially available product. The IDS will dispense coded, blinded study drug kits to the research coordinator at each site. Starter kits containing three weeks of study medication will be following by three other kits, 2 of which will contain 4 weeks of study medication (for week 4-7 and for weeks 8-11)and one of which will contain 5 weeks of study medication (for weeks 12-16). Each kit will contain medication divided into weekly prescriptions that contain 9 days of medication (i.e., an extra two days of medication (i.e., 4 capsules) to ensure that subjects have adequate medication to continue the prescribed regimen if a visit needs to be rescheduled). The research coordinator will maintain 100% drug accountability at the clinic for the duration of the study. The research coordinator will document all medication using a drug accountability log to track the study medication from time it leaves the IDS until the time that it is returned to the IDS for reconciliation and disposal.

5.9.2 Storage

Study drug will be stored at the IDS at controlled room temperature (36-86° F). Study drug kits dispensed from the UMB IDS to the clinics at UPenn and UMB will be stored in a locked cabinet in a room that is temperature controlled and double locked until dispensed. Any returned study medication will also be kept under locked conditions until returned to the UMB IDS for disposal.

5.9.3 Dispensing of Study Drug

Upon receipt of the Subject Randomization Form for a particular participant, the study staff will have the medication for that subject ready for dispensing. Medication will be dispensed to subjects at each study visit, with enough medication provided to last until the next scheduled visit (with two days of additional medication to ensure that the subject has adequate medication if the appointment is not scheduled for the exact number of weeks or has to be rescheduled). All clinical staff members directly involved with the assessment of subjects are always blind to the medication condition. These procedures have been designed to avoid delay. The randomization and assignment of eligible subjects into groups will be conducted at by an individual who is not otherwise involved in the study. All participants and other investigators will be blinded.

The study nurse and study coordinator will maintain a dispensing record, documenting the amount of medication dispensed to the subject, the amount returned, and the amount taken. A log will be used to document the amount of study drug returned to the IDS and the date it was returned.

5.9.4 Return or Destruction of Study Drug

A final reconciliation of all remaining study medication will be made at the end of the study. All unused kits and medications dispensed and later returned by the subjects will be returned to the UMB IDS, which will be responsible for the disposal/ destruction of all unused study medication. The study coordinator will reconcile all remaining study medication for each subject as they complete the study and return all unused study medication and empty medication bottles to the UMB IDS for reconciliation in batches on a quarterly basis. All attempts will be made to collect the study medication and bottles from subjects at the end of the study and during follow-up sessions as needed.

6 Study Procedures

6.1 *Initial Telephone Screen (*approximately 20 minutes)

Subjects will be recruited through local advertisements in print and broadcast media, through leaflets and posters distributed throughout the Greater Baltimore regions, and by advertising on-line and through social media, and by communicating with them by mail (as described in section 4.1). Subjects who express interest by responding to this advertising will be contacted to participate in a telephone screening interview. Prior to eliciting any information, we will describe the nature of the study, including the number of visits to the center, subject protections and confidentiality. If the caller is still interested, we will obtain oral consent for the phone screening. Screening questions cover general health, psychiatric history and alcohol consumption. If, in consultation with the principal investigator or his designee, we determine that the applicant is eligible based on this screening, s/he will be invited to come to the clinic for an in-person screen visit.

6.2 Screen Visit (V0-1; Study Week -1)

This visit is designed for genetic screening to allow stratification on the tested genotypes Upon arriving to the visit, which will be conducted at the CNC in Columbia, MD, subjects will be asked to show legal photo identification and to provide informed consent. They will then undergo a breathalyzer test to ensure a breath alcohol concentration (BrAC) of 0.00%, which will be used to assess alcohol intoxication at every visit. The allowable alcohol concentration at all visits following the screening visit is less or equal to 0.02. The informed consent will be considered valid and no assessments will be performed at the screening or any subsequent visit unless subjects have BrAC readings of 0.00%

If the subject attends the clinic and records a BrAC greater than this level, the study staff will encourage the subject to wait in the clinic for a sufficient time to permit testing. If a subject coming to the UMB site records a BrAC level >0.02 mg/dl , and does not agree to wait, or it is impractical for the subject to wait until the BrAC falls to the desired level for testing, he or she should be advised not to leave until the BrAC is below the legal level. If the subject insists on leaving at a level at or above the legal limit, this should be documented in the appropriate section of the CRF. The investigator should make a reasonable effort to reach the subject after he or she leaves the clinic to ascertain his or her safety and to encourage him or her to attend the next clinic visit.

The screening visit can be done in one or more visits, depending on participant's availability and convenience. If necessary, because of technical issues preventing a valid test or for clinical considerations, we will allow repeating any screening clinical laboratory test at least twice (at the same or future visit).

Subjects will be given the informed consent form to read, after which a study staff member will review it with the subject, providing an explanation of the study protocol, its risks, potential benefits, and alternative treatments. Following resolution of any questions, subjects will be asked to sign the study informed consent form. An entire signed copy of the informed consent form will be given to the subject, who will be reminded that the consent addresses his or her willingness to participate but that the subsequent screening process will determine his or her eligibility to do so. Further, the subject will be reminded that participation is voluntary, and at any time, he or she may withdraw from the study.

The study personnel will inform the subject that the purpose of this visit is to determine Eligibility based on whether or not it is medically safe for them to participate. The study personnel will also describe the Brief Behavioral Compliance Enhancement Treatment (BBCET; see section 6.8, below), the psychosocial treatment that will be employed for this study.

The Screen Part-1 Visit will consist of the following:

- BrAC, vital signs ,body weight, and height
- Obtain informed consent (signature by subject)
- Race/Ethnicity questionnaire
- Demographics
- Names and addresses of one or two locators (people who will know the whereabouts of the subject to assist in locating him/her during treatment, if needed)
- Medical/Psychiatric history and physical exam
- Electrocardiogram (ECG)
    - Blood collection – 45 mL for:
    - Hematology and clinical chemistry tests, DNA extraction and genotyping
    - Gamma-glutamyl transferase (GGT) to assess the validity of self-reported drinking.
    - Carbohydrate-deficient transferrin (%dCDT; to assess the validity of self-reported drinking)
    - RNA and miRNA expression studies(To be stored at -80^{0}C for future studies)
- Urine collection for
    - Urine toxicology (presence of psychoactive drugs)
    - Urinalysis
    - Urine pregnancy test (for women of child bearing potential)
- Complete assigned questionnaires and rating scales (see section 6.7, below)
    - SORT
    - AUDIT
    - SCID-IV with the Alcohol Use Disorder module to include DSM-5 criteria
    - SSADDA Nicotine Dependence Module
    - TLFB (for 90 days prior to the visit)
    - CIWA-Ar
    - MINI Suicide Risk Assessment Scale (every visit)

If the Principal Investigator (or designee) deems it necessary, all or part of any screening assessment or laboratory test may be repeated up to two times (at the same or a future visit), if needed because of technical issues preventing a valid result or for clinical considerations.

6.3 Baseline Visit (Week 0)

Subjects who have one of the specific desired genotype combinations will be notified of that fact within one week and asked to return to the CNC within 30 days from screening visit for additional screening procedures. If the subject continues to be eligible, he/she will be randomized and will receive the first dose of ondansetron or placebo based on their assigned randomization schedule. The baseline visit can be spread out over 2 visits, depending on participant's availability and convenience.

The Baseline Visit will consist of the following:

<u>Before the first dose of ondansetron:</u>

- BrAC, weight and vital signs
- Urine collection for:
  - Riboflavin measurement for compliance
  - Urine pregnancy test
- Complete assigned questionnaires and rating scales(see section 6.7 below)
  - TLFB
  - CIWA-Ar
  - Other Drug Use questionnaire
  - SF-12
  - SIP
  - ADS
  - OCDS
  - BDI
  - PSQI
  - MINI Suicide Risk Assessment Scale (every visit)
  - CGI-I and CGI-S
  - Administer BBCET
- Names and addresses of two locators (people who will know the whereabouts of the subject to assist in locating the subject during treatment, if needed
- Review of eligibility
- Record medication changes and treatment history since screening

NB: If an abnormality is found during screening that could pose a risk to the subject, prevent the subject from completing study procedures, or interfere with study results, the investigator may decide not to administer study medication.

Once it has been determined that the subject meets all inclusion and no exclusion criteria, the study nurse or physician will administer first dose of study medication and dispense first week of medication to subject, giving instructions on how to take the medication twice daily for 16 weeks. Study nurses will instruct subjects to call at any time to discuss any problems or concerns that they may have while taking the study medication.

<u>After the first dose of ondansetron</u>

- BBCET (see section 6.8 below) will be administered.

6.4 On-Treatment Visits (Weeks 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, and 20)

Subjects will be seen at every week (± 4 days) for the first six weeks, every two weeks (± 4 days) from weeks 6-12 and 4 weeks following week 12, at week 16. At the baseline, week 4, and week 8 visits, a dose of the study medication will be administered in the clinic. The following study procedures will be performed at the on-treatment visits.

- BrAC , body weight, and vital signs (every visit)
- Blood sample: 15 mL for GGT, %dCDT (Week 8) and 15 ml for future RNA and miRNA expression studies (weeks 4, 8 and 12) as close as possible to the same time of day
- Urine to screen for drugs of abuse (Week 8), pregnancy test (for women of child bearing potential) (Weeks 4, 8, 16, and 12) and riboflavin measurement for compliance (every visit)
- ECG (visit 5 only)
- Other Drug Use questionnaire
- Administer a scheduled dose of medication (first or second of the day) in the clinic (weeks 4 and 8)
- Complete questionnaires and rating scales (see section 6.7 below)
    - TLFB (every visit)
    - CIWA-Ar (every visit)
    - MINI Suicide Risk Assessment Scale (every visit)
    - OCDS (every visit)
    - BDI (every visit)
    - PSQI (every visit)
    - CGI-I and CGI_S (every visit)
    - SF-12 (week 8)
    - SIP (week 8)
    - ADS (week 8)
- Administer BBCET (every visit)
- Record concomitant medication and treatment use since last visit (every visit)
- Document study medication compliance (every visit)
- Record adverse events

If a subject stops study medication at any time during the study, he/she will be encouraged to continue to attend study visits and participate in all non-study-drug-related procedures of the study.

6.5 End of Study Visit (Week 16)/Early Termination Visit

The subject will return to the CNC at Week 16 (±4 days) for the End of Study visit. Subject will have completed 16 weeks.
of study medication prior to the last visit.

<u>The following procedures will be performed at the Week 24 (End of Study) visit:</u>

- BrAC, body weight and vital signs

- Blood sample – 15 mL for GGT, %dCDT, hematology and clinical chemistry tests and 15 ml for RNA and miRNA extractions (this sample will not be obtained at an early termination visit)
- Physical examination
- Urine pregnancy test (for women of child bearing potential) and urinalysis
- DSM-5 AUD criteria
- Complete all assigned questionnaires and rating scales (see section 6.7 below)
    - CIWA-Ar
    - TLFB
    - SIP
    - OCDS
    - BDI
    - PSQI
    - CGI-I and CGI-S
    - SF-12
    - ADS
    - MINI Suicide Risk Assessment Scale
- BBCET session
- Record concomitant medication and treatment use since screening
- Document compliance
  Record adverse eventsThis visit should be conducted not only for all subjects who complete the treatment but those who discontinue from the study early as well.

6.6. Safety Follow-up Visit (Week 20)

At this visit, a BrAC and vital signs will be measured and study personnel will assess persistent adverse events following the cessation of study medication. If the subject discontinues the study early, the visit can be by telephone unless an SAE occurred during the study and it has not been fully resolved by Week 16. Individuals who complete the study medication period will be invited to give an optional blood draw for mRNA and miRNA expression studies.

6.7. Assessments

- Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (SCID-IV). Diagnosis by trained clinicians will be based on DSM-IV diagnoses (American Psychiatric Association, 1994) except for the alcohol use disorder diagnosis, which will be based on the 5th edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) (American Psychiatric Association, 2013). For the AUD diagnosis, we will adapt the Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders* (SCID-IV) (First, Spitzer, Gibbon, & Williams, 1994), to ensure that subjects meet at least two of the 11 diagnostic criteria. The SCID will also be used to exclude subjects with exclusionary psychiatric diagnoses.
    - The Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA). The SSADDA is a polydiagnostic instrument developed for use in genetic studies of alcohol and drug dependence. Because the SCID-IV does not assess nicotine dependence, the Nicotine dependence module of SSADDA interview will be used to characterize the study sample with respect to a DSM-IV diagnosis of nicotine independence.
- Alcohol Dependence Scale (ADS total score). The ADS was designed to provide a quantitative measure of the severity of alcohol dependence consistent with the concept of the alcohol dependence syndrome. The Alcohol Dependence Scale is a rater-administered, 25-

item questionnaire covering alcohol withdrawal symptoms, impaired control over drinking, awareness of a compulsion to drink, increased tolerance to alcohol, and drink seeking behavior. The ADS will be administered by a trained member of the research team.

- The Alcohol Use Disorders Identification Test (AUDIT) will be used to screen potential study participants for alcohol use and related problems. The AUDIT is a 10-item self-report questionnaire that measures alcohol consumption, dependence symptoms, and personal and social harm reflective of drinking. The AUDIT has demonstrated good content, criterion and construct validity (Babor, de la Fuente, Saunders, & Grant, 1992), and reliability (Cronbach's alphas ranging from 0.77 to 0.83) (Bohn, Babor, & Kranzler, 1995). The AUDIT will be administered at the site as part of the telephone screening interview.

- The Timeline Follow-back (TLFB) method to quantify alcohol consumption will be administered at every study visit by a trained member of the study team. The TLFB is a daily calendar for alcohol consumption that employs memory aids, such as a calendar, to help subjects provide retrospective estimates of their daily drinking as the number of standard drinks for each day. A day is defined as a 24-hour period starting at 6.00 a.m. and ending at 6.00 a.m. the following morning. A month is a period of 28 consecutive days. The TLFB can be completed in a reasonable length of time (approximately 10-15 min) and has been used extensively in pharmacotherapy trials for alcohol dependence. At the Baseline Visit, the TLFB will be used to measure alcohol consumption for the prior 28 days. At each subsequent visit, the TLFB will be used to obtain alcohol consumption since the prior visit. If the subject misses a scheduled visit and returns for a later visit, the TLFB will be administered to document alcohol consumption during the missed period. For example, if the subject attends Visit 6 (Week 4), does not attend Visit 7 (Week 5) and returns to the study center for Visit 8 (Week 6), the TLFB administered at Visit 8 will elicit consumption data for the period of time since the Visit 6 TLFB administration. If the subject has not withdrawn consent for participation in the study, every effort should be made to conduct a Week 28 visit. If the subject refuses to participate in an on-site study visit, the study center may administer the TLFB via phone, at Week 20 to obtain the missing data.

- Mini International Psychiatric Interview (MINI) Suicide Risk Assessment Scale (Module B) is a subscale of the MINI neuropsychiatric interview. It has been shown to help identify patients at risk for self-harm.

- Obsessive Compulsive Drinking Scale (OCDS) is a 14-item, self-report measure that assesses aspects of alcohol craving, including obsessive thoughts about alcohol use and compulsive behaviors toward drinking.

- Revised Clinical Institute Withdrawal Assessment for Alcohol scale (CIWA-Ar) consists of 10 items, 9 of which (nausea and vomiting, tremor, paroxysmal sweats, anxiety, agitation, tactile disturbances, auditory disturbances, visual disturbances, headache, fullness in head) are rated on a scale of from 0 to 7. The 10th item (orientation and clouding of sensorium) is rated on a scale of from 0-4. It takes approximately 2 minutes to administer and score the CIWA-Ar. The CIWA-Ar scale must be administered by the investigator or another qualified member of the research team. The research team member who administers the CIWA-Ar assessment will be trained in the scoring conventions for the scale.

- Clinical Global Impression-Improvement Scale (CGI-I The CGI-I is rated on a 7-point scale: 1=very much improved; 2=much improved; 3=minimally improved; 4=no change; 5=minimally worse; 6=much worse; or 7=very much worse and permits a global evaluation of the subject's improvement (or worsening) over time. The clinician is required to assess the subject's condition relative to baseline at every study visit. In all cases, the assessment should be made independent of whether the rater believes the improvement is drug-related or not. CGI-1 will be administered by the investigator or other qualified member of the research team, after training. Due to the simplicity of the scale, the CGI will be used in the original English version.

- Clinical Global Impression-Severity Scale (CGI-S) The CGI-S assesses the clinician's impression of the subject's current clinical condition, and is rated on a 7-point scale: 1= normal, not at all ill; 2= borderline ill; 3= mildly ill; 4= moderately ill; 5= markedly ill; 6= severely ill; 7= among the most extremely ill subjects. The clinician should use his/her total clinical experience with this subject population and rate the current severity of the subject's clinical condition on a 7-point scale. CGI-S will be administered by the investigator or other trained, qualified member of the research team. Short Index of problems (SIP) is a 15-item self-report instrument
that will be administered at the screening, baseline, Week 4, Week 8, Week 12and Week 16 (End of Study) visits. The SIP measures specific and direct harmful consequences of drinking. Each item is assessed on a 3-point scale ranging from "never or once or a few times" to "daily or almost every day." A lower score indicates fewer adverse consequences of drinking. The DrInC, which contains items that measure a wide range of alcohol-related problems (including alcohol dependence symptoms and medical, psychological, social, occupational, and legal problems), has been shown to be a reliable and valid measure of alcohol-related problems. The SIP is simpler to administer and, like the DrInC, has been shown to measure a single factor of alcohol-related problems.

- Beck Depression Inventory (BDI) The 21-item BDI will be used to measure depressive symptoms. Questions focus on topics such as mood, thoughts of suicide, sleep habits, and eating behaviors. The BDI will be used to assess the severity of any depressive symptoms, particularly suicidal risk. The BDI will be administered at every study visit. At screening/baseline, subjects with severe, untreated depression or who endorse suicidal intent will be excluded from the study and referred for treatment or immediate intervention. At each post-baseline visit, the staff will review the findings on the BDI to ensure that the subject's safety is not compromised by changes in depression symptoms.

- Pittsburgh Sleep Quality Index (PSQI) is a self-rated questionnaire assessing sleep quality and disturbances during a 1-month period. It contains 19 items that generate 7 component scores on subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction, all of which add up to a global score. Because sleep disturbance is common in alcoholism, recovery may be associated with improved sleep.

- Other Drug Use questionnaire is a brief self-report questionnaire on which the subject is asked to list his/her use of any prescribed or non-prescribed drug use occurring since the last study visit. Upon its completion, a study staff member reviews the questionnaire and obtains details about any reported drug use.

- RAND 12-Item Health Survey (SF-12), a self-report measure of life functioning in various domains. It yields an overall health-related quality of life score and physical and mental health component summary scales.

6.8 Brief Behavioral Compliance Enhancement Treatment (BBCET)

Delivery of BBCET: BBCET is the psychosocial adjunct intervention for the study. A BBCET manual has been developed by the UMB group. A BBCET-trained staff member from UMB will train the BBCET provider(s) at the UPenn site and will meet monthly by telephone with the staff members who administer the BBCET to address any issues. BBCET sessions shall occur at every study visit and will be delivered by a trained nurse or masters level clinician.

All BBCET sessions will be audiotaped. Study staff will not use names or disclose PHI during the audio-taped sessions. Study staff will identify the subject by study ID and the audio recording will be uploaded to a secure server until reviewed by study staff (exclusively) and then deleted. The BBCET supervisors will review 10% of these tapes to ensure adherence to its

uniform delivery. Including the pre-randomization and termination BBCET sessions, there will be a total of 11 BBCET sessions for the 16-week trial. Each BBCET session lasts about 10–15 minutes and is delivered contemporaneously with the accounting of the amount of study medication taken from the last visit and the ascertainment of adverse events. BBCET sessions focus on compliance with the medication and reinforce adherence to the protocol. BBCET sessions also reinforce the importance of taking study medication and provide general motivation, encouragement, and support for continuing in treatment. BBCET provides education on the negative effects of excessive alcohol use on physical and mental health. The rationale for using the medication is discussed in simple language. Physical examination and laboratory results are discussed with each participant. BBCET helps subjects to overcome ambivalence, which may interfere with their desire to change. Adherence to medication and compliance with the protocol are praised and reinforced.

BBCET emphasizes that alcohol dependence is a disease process that can be tackled by compliance with the study regimen. BBCET mimics a naturalistic doctor/patient relationship in function and duration and will feel familiar to most participants. BBCET does not allow for the interpretation of feelings, psychological interpretations about the nature of alcohol dependence, or applying problem-solving techniques to matters other than medication compliance. One hallmark of BBCET is that subjects set their target drinking goal for the next period at each session. BBCET can be more readily generalized than formal psychotherapies for use in generic treatment or family practitioner settings because it is much briefer. General health care workers can be trained to deliver it, thereby increasing greatly clinical access to care for the treatment of alcohol dependence. BBCET has been used successfully in previous pharmacotherapy trials.

BBCET is delivered in 3 phases.

In Phase 1 (Baseline and Weeks 1–3), the BBCET provider builds rapport with the subject to inspire confidence, trust, and warmth, discusses medication effects, instructs on the importance of taking the medication regularly, provides a basic and easily understandable model of how the medication(s) might work, addresses concerns about taking medication, adverse events, and compliance barriers with the subject in understandable terms, and clearly conveys his or her knowledge and experience with the pharmacotherapy of alcohol problems. The BBCET provider allows the subject to air prejudices, corrects any misconceptions (which may include “all or none” expectations), and provides a medication information sheet with an emergency contact card that describes the possible medication being taken and appropriate contact numbers for the investigator or study staff.

In Phase 2 (Weeks 4–10), the emphasis is on maintaining compliance and avoiding early discontinuation of the medication. Adverse events are monitored and protocol-permitted remedies or over-the-counter medications are provided if necessary to deal with them.

In Phase 3 (Weeks 12 and 16), the BBCET provider and the subject discuss the subject’s experience throughout the study. Typically, the subject will be offered referrals, and the termination visit will take place.

The BBCET provider will discuss plans regarding how to discontinue the medication and will provide a rationale of how the medication may have helped the participant to achieve his or her goal. It is expected that a significant provider-subject relationship has been developed during the study, and care should be taken in the termination of this relationship.

Subject Compliance with BBCET Administration: BBCET is the only allowed psychosocial treatment. Subject participation in BBCET sessions will be tracked. Evidence of non-compliance could result in discontinuation of the subject from the study.

Practitioner Adherence to BBCET: BBCET sessions will be audiotaped. A random selection of 10% of the tapes will be reviewed at each site to ensure adherence to the protocol. Recertification and/or retraining of those administering BBCET will be used to correct any

protocol deviations. A provider checklist (PSC) will be administered as an additional check on compliance.

6.9 Genetic Evaluation

Blood sample collection: Two vaccutainer tubes containing ACD buffer (to prevent coagulation) will be used to collect a total of 15 ml of peripheral venous whole blood from each subject as shown in Table 1 . All blood samples collected for DNA, mRNA and miRNA extraction will be processed and stored at 4°C to be sent to Dr. Seneviratne's laboratory at the UMB Institute for Genome Sciences (IGS).

Genotyping for subject stratification and randomization: All genotyping for the subjects enrolled at both UMB and UPenn will be performed at the IGS. Genomic DNA will be extracted from whole blood samples using established procedures from Dr. Seneviratne's previous work (Ait-Daoud et al., 2009; Johnson et al., 2011; Seneviratne et al., 2009).

The 5-HTTLPR genotypes LL, LS and SS and the rs25531 genotypes AA, AG and GG will be will be performed simultaneously using restriction fragment-length polymorphism (RFLP) method. Genotyping for the SNPs of interest (listed below) will be performed with commercially available TaqMan® allelic discrimination assays (Applied Biosystems, Foster City, California). Applied Biosystems 7900HT Fast Real-Time PCR system will be used for amplification and capture of the resulting florescent signals.

- rs1042173 (TT, TG, and GG genotypes) in the 3′-untranslated region of SLC6A4 that encodes the serotonin transporter;
- rs1150226 (AA, AG and GG genotypes) in the 5′UTR of HTR3A gene that encodes subtype A of the serotonin-3 receptor;
- rs1176713 (GG, GA and AA genotypes), a synonymous SNP in exon 9 of HTR3A gene that encodes subtype A of the serotonin-3 receptor;
- rs17614942 (AA, AC and CC genotypes), an intron 8 SNP in HTR3B gene that encodes subtype B of the serotonin-3 receptor
- rs1176744 (AA, AC, CC genotypes), an exon 5SNP in HTR3B gene that encodes subtype B of the serotonin-3 receptor (for UPENN site only)

All samples will be genotyped in duplicate and any discrepancies will be repeated to ensure the accuracy of the procedure. No results of genetic testing (including ancestry genetic panel, if done) will be provided to participants, even if they request it. A requesting participant will be reminded that the informed consent form stated that no genetic test results would be provided to participants.

mRNA and miRNA expression studies: The total RNA extractions will be performed after completing the entire proposed study, using supplemental funding. Until then, the blood samples will be stored securely in -80^{0}C freezers. The genome-wide expression levels of mRNA and miRNA in WBC (cellular mRNA and miRNA) as well as circulating miRNA in serum will be quantified with next generation sequencing based technologies available at the sequencing core of the IGS.

6.10 Missed Visit

If a subject anticipates missing a study visit (e.g., due to a vacation), the study nurse will provide the subject with enough study medication to ensure the maintenance of the daily dosage of study medication until the next scheduled visit as per the dosing schedule, either in person or via mail. Subjects will be instructed to call the UPENN study staff at any time to discuss problems or concerns that they may have while participating in the study. They will be provided with phone numbers to contact the study staff during office hours and a pager number for off-

hours contact. Subjects will be asked to come in for their next study visit as soon as possible. A subject unable to attend an in-person visit will be asked to provide all scheduled assessment information by telephone. A study physician will approve all study medication mailed to the subjects.

If a subject calls to cancel a scheduled study visit due to an unforeseen event, the study visit will be rescheduled to occur as soon thereafter as possible. Study staff will send study medication to the subject via overnight mail under the supervision of a study nurse to avoid having the subject run out of study medication. Subjects will be given only enough study medication to ensure that they maintain their daily dosage of study medication until their next scheduled visit. The subject will be asked to record any adverse events that may have occurred since the last study visit. Study nurses will instruct subjects to call at any time to discuss any problems or concerns that they may have while taking the study medication. A study physician will determine how many additional weeks of study medication a subject will be allowed to receive without attending a study visit and will approve the mailing of all study medication.

7 Statistical Plan

7.1 Sample Size Determination

For the 2 EA genotype groups discussed above (section 3.1), we are expecting that 61% of the EA sample will be in the responsive genotype strata and 29% of the EA sample will be in the non- responsive genotype strata based on prior studies[5]. Block randomization will balance allocation of the 2 treatment conditions (within each racial group) with the responsive and non-responsive genotype strata. Hence, after 30% dropout, the expected cell frequency for the 2 treatment arms for EAs will each be 39 in the responsive strata and 25 in the non-responsive strata. For two of the AA genotype grouping discussed above (section 3.1), we are expecting that the 5-HTTLPR L_AL_A group, which in the randomized sample is estimated to include 55 AAs subjects (i.e., ~30% of the total randomized sample based on the frequency of the L_AL_A genotype in AAs) and subjects with rs1042173*TT genotype (which is estimated to include 106 subjects, i.e., 58% of AAs have the TT genotype). Power analysis was done using the methods in Section 8.3 in Cohen (1988). For the power calculations, we estimated that the effect size for reduction of DDD between the ondansetron and placebo groups in the responsive genotype group would be 0.59[5]. Assuming an alpha level of 0.05 and a sample size of 39 in each arm we will have power equal to 0.84. Because the intent-to-treat principle on which our analysis is based includes all randomized subjects in the statistical analyses, and because we will use enhanced techniques to ensure that we obtain most of the drinking data, our study has more than adequate power to test the stated hypotheses.

We will also test whether ondansetron is better than placebo overall (i.e. regardless of genotype). For this comparison we expect to have 64 EA subjects in each treatment arm and 64 AA subjects in each treatment arm. With alpha=.05, we have power equal to .81 to detect effect sizes down to .44.

7.2 Statistical Methods

We expect to have n=128 treatment-seeking alcohol dependent individuals who complete the 16 week trial at each site. However, we will include all randomized subjects in the

main intent-to-treat analyses. Stratified randomization will ensure approximate balance between the 2 treatment conditions (ondansetron and placebo) within genotype strata (as discussed above) Group assignment will be achieved using **a permuted block randomization procedure** that further balances the treatment groups on study site, race and gender. Statisticians who will have no contact with subjects or with clinical staff involved in direct patient care have created the randomization list which will be used by the UMB Investigational Drug Services. Subjects also will receive Brief Behavioral Compliance Enhancement Treatment (BBCET) as their psychosocial adjunct weekly in weeks 1–8 and then every 2 weeks in weeks 8-16.

Descriptive summaries and group comparisons: Data will be summarized for the primary and secondary outcome measures, demographics, and baseline characteristics. Generally, quantitative variables will be summarized with standard descriptive statistics (e.g., range, quartiles, means, and standard deviations), while frequency tables will summarize categorical variables. Group comparison for quantitative variables will be done using standard two-sample *t*-tests or analysis of variance as appropriate. Highly skewed variables may be transformed before inferential comparison, or non-parametric methods may be used. Group comparison for categorical variables will be analyzed with two-sample proportional comparisons or chi-square tests as appropriate.

Analyses of primary and secondary outcomes: The primary efficacy variable will be change from baseline in DDD. Secondary outcome variables will include other measures of drinking—percent heavy drinking days (PHDD) and the percentage of days abstinent. Exploratory drinking outcomes will include the novel measure—percentage of subjects with no heavy drinking days (Falk et al., 2010), which presently is the standard identified by the FDA as an outcome measure for registration trials in alcohol dependence.

The general approach to statistical analyses will be the same as that utilized in our previous studies (Johnson et al., 2011; Johnson et al., 2000). **All randomized subjects will be included in the analysis using the intent-to-treat principle.** We will use a mixed-models approach, adjusting for **baseline covariates (when significantly different by group) and the same drinking variables for baseline**. For the drinking variables, the baseline value is the average for the 90-day period prior to the screening visit. Other potential covariates shall include age, race, and sex. In the first model, we will assess whether individuals who are hypothesized to be in the responsive genotype group and treated with ondansetron will have greater reduction in DDD than the corresponding placebo group. In the second model, we will test whether EA subjects who are treated with ondansetron will show fewer DDD than the corresponding placebo group overall (regardless of genotype). We also will explore the 'difference in differences' hypothesis that the difference in DDD will be greater in the hypothesized responsive genotype group than the difference in the hypothesized non-responsive genotype group, however the study is not powered for this. The residuals from our analyses will be checked for normality by computing their skewness and kurtosis and will be checked for homogeneity of variance by plotting them in a histogram and against predicted outcomes. Standard (i.e., square root or log) transformations will be employed to improve the distributions when required.

Before we conduct the analyses, the groups will be compared to verify balance on the age, gender, and drinking severity variables, as well as other important baseline demographics and clinical characteristics. If, as expected, there are no significant group differences, these baseline and balancing variables will be dropped from further analyses. Possible (though unexpected) group differences will be further examined and interpreted as to their clinical meaning and whether they may confound the interpretation of planned comparisons. Since this is a simple factorial model, the interpretation of main effects and their interaction is straightforward. Where there is a significant main effect for an independent variable with three or more levels or an interaction, post-hoc comparisons will be done.

The mixed-models approach is our first analytic choice as it assumes a random effect for missing data. If, however, there is a differential attrition rate (i.e., if dropout is informative), we shall consider other approaches (e.g., joint models of longitudinal drinking outcome and time to dropout) to account for informative dropout to obtain valid results (Liu, 2009; Wulfsohn & Tsiatis, 1997). Statistics will be run using SAS 9.1 (SAS Institute Inc., Cary, NC).

In secondary analyses we will conduct analyses similar to the main analyses only with change from baseline in number of heavy drinking days (HDDs) during the last 8 weeks of treatment as the outcome variable. We will also conduct a responders analysis on this secondary outcome with endpoint: no heavy drinking days over the last 8 weeks of treatment versus one or more heavy drinking days in the last 8 weeks of treatment. For this outcome we will use an ordinary logistic regression model to compare the treatment groups in the whole sample and within the hypothesized responsive genotype adjusting for the same covariates as in the main model. One additional alternative endpoint that we will examine is mean daily alcohol consumption (in standard drinks/day) over the last 4 weeks of the 16-week treatment period. This will be analyzed with ordinary linear regression for a continuous outcome.

As an exploratory procedure, we also will use the more advanced two-part random effects model (Olsen & Schafer, 2001; Tooze, Grunwald, & Jones, 2002) to analyze the daily drinking record. This model can simultaneously characterize the frequency (odds of daily drinking being zero) and quantity (the number of drinks on a drinking day) of drinking. It is more efficient and can tackle non-normality in the drinking level. We also will do trajectory analyses of different treatments over time to capture differences in drinking outcomes (Chen et al., 2011). Such an analysis is more clinically intuitive and could be more powerful than the analysis based on endpoints.

Furthermore, as an additional exploratory step to provide information on whether ondansetron treatment has differential efficacy for specific genotypes in a “relapse prevention” model rather than with the “currently drinking” approach, we will do secondary analyses on those who quit drinking during the study to determine whether, and by how much, these results differ from our primary analyses. We will report and discuss these secondary analyses along with the primary approach in our publications.

7.3 Subject Population(s) for Analysis

The primary efficacy analysis will be conducted with the intent-to-treat population consisting of all individuals who are randomized to treatment and take at least one dose of study medication. This will require at least one post-randomization measurement of outcomes. Similarly, all safety evaluations will include all available subjects on whom any safety information is obtained.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others: Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

- <u>Unexpected in nature, severity, or frequency</u> (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- <u>Related or possibly related to participation in the research</u> (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)

- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

An Adverse Event (AE): is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

A Serious Adverse Event (SAE): Any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period: The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition: A pre-existing condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings: At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event: All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The

sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values: A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery: Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others

(see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.3.1 Investigator reporting: notifying the study sponsor

Each study site will be responsible for reporting any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, and all other adverse events as required by the UMB/UPENN IRB sites (see sections 8.3.2) to the study sponsor (NIAAA) and FDA. Both IRB sites will be notified of all reported events and outcomes at both sites. The investigator will keep a copy of these reportson file at the study site.

Within the following 48 hours, the investigator must provide further information on the serious adverse event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to both sites.

8.3.2. Investigator reporting: notifying the Institutional Review Board

This section describes the requirements for safety reporting by investigators who are UMB/Penn faculty, affiliated with a UMB/Penn research site, or otherwise responsible for safety reporting to the IRB. The Institutional Review Boards at UPenn and UMB require expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The IRB requires researchers to submit reports of the following problems <u>within 5 business days from the time the investigator becomes aware of the event:</u>

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

  <u>Unexpected</u> (An event is “unexpected” when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

AND

Related to the research procedures (An event is "related to the research procedures" if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)

Reporting Process: Unanticipated problems posing risks to subjects or others as noted above will be reported to the UMB IRB in CICERO under reportable new information within 5 business days.

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Reporting Deaths: more rapid reporting requirements

Deaths that occur during the course of a research study and that are:

1) Unexpected; AND
2) Related to the research study; AND
3) When other participants are believed to be at an increased risk of harm

Must be reported to the IRB within 3 business days from the time the investigator becomes aware of the death.

Other Reportable events: For clinical drug trials, the following events are also reportable to the IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
- An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
- Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
- A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
  - Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
  - Breach of confidentiality
  - Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
  - Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
  - Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
  - Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

8.3.3 **Sponsor reporting: Notifying the FDA:**

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

– ***Within 7 calendar days***

Any study event that is:

– associated with the use of the study drug
– unexpected,
– fatal or life-threatening, and

– ***Within 15 calendar days***

Any study event that is:

– associated with the use of the study drug,
– unexpected, and
– serious, but not fatal or life-threatening

-or-

- a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

Any finding from tests in laboratory animals that:

– suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements: Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process: Adverse events may be submitted on FDA Form 3500A or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3.

8.3.4 Sponsor reporting: Notifying participating investigators

It is the responsibility of the study sponsor to notify all participating investigators, in a written IND safety report, of any adverse event associated with the use of the drug that is both serious and unexpected, as well as any finding from tests in laboratory animals that suggest a significant risk for human subjects. Additionally, sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

8.4 Unblinding Procedures

On the day of randomization, participants are given an emergency notification card. The emergency notification card has a 24-hour emergency number by which to contact a PI or

physician co-investigator in case of an after-hours emergency. At UPenn, the number is a pager that is in the possession of one of the study physicians. The coverage physician will then be responsible to contact the participant to determine the nature of the emergency and to provide reassurance, advice, or treatment referrals as appropriate. In the morning, the study physician is responsible to inform the PI, document the event, and follow-up with the participant.

Only the PI or another study physician is authorized to break the blind dose code, which will be done in collaboration with the IDS at UMB, where the randomization information will be retained. The decision to break the study blind for an individual subject lies with the study PI (or a designated co-investigator if a PI is not available) and would be resorted to only in cases of anemergency when knowledge of the treatment arm investigational agent will influence clinical management.

8.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.5.1 Data and Safety Monitoring Board

The DSMB will include relevant experts at UPenn. The DSMB members will include James McKay, Ph.D. (chair), Kevin Lynch, Ph.D. (biostatistician), Richard Feinn, Ph.D., David Metzger, Ph.D., Daniel Weintraub, M.D., Deb Dunbar, MSN, CRNP, and Cynthia Clark, Ph.D. The DSMB will meet by teleconference annually or more often if warranted to review progress pursuant to presentation of a summary report from the principal investigator, ensuring that policies on the identification and reporting of adverse events to the appropriate regulatory bodies, which can include the local IRB, the FDA, and the project officer at the NIH, have been implemented diligently and promptly. Prompt and due diligence reporting of adverse events (i.e., within 24 hours during the week and on the next working day following a weekend) to the regulatory bodies remains the express duty of the principal investigator. The DSMB will retain the right to make independent representation to the regulatory bodies if there has been a failure or lapse in reporting by the principal investigator. The DSMB also will have the capacity to instruct the principal investigator to pause or terminate pursuance of research if regulatory body guidelines are contravened. The DSMB will provide regular independent reports to the IRB concerning the protection of human subjects in this study. These DSMB procedures have been approved previously by the NIH for our ongoing clinical studies.

Frequency of data and safety monitoring reviews: These investigators have extensive experience with clinical trials using pharmacotherapy for the treatment of substance abuse and with the recruitment and retention of substance-dependent subjects. In the past, such trials have been done with minimal risks to participants. Therefore, the DSMP will include two items: The research members at each site, including the PI, co-investigators, research nurses, and project coordinators, will meet weekly to discuss progress on the study and to address any issues related to the research procedures. The DSMB will meet annually and will generate a report that is sent to the PI at each site. The report will summarize the Board's review of cumulative serious and unexpected adverse events and any other concerns that may arise.

Content of data and safety monitoring report: The PI at each university will be responsible to monitor the safety and efficacy of this trial, executing the DSMP, providing the DSMB with the needed information, and complying with the reporting requirements. The overall PI will provide a summary of the data and safety monitoring report to staff at the NIAAA on an annual basis as part of the progress report.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Records, filed in the IRB office, verify that all research project personnel have completed training in the protection of human research subjects in accordance with the guidelines of the U.S. Department of Health and Human Services (DHHS) and the Office for Human Research Protection (OHRP). The study staff (PI, Clinical research coordinator, etc.) will keep all study medical records (including any codes to de-identified data) under lock and key in a secure location, as required by law. Only date and time of the research visit and lab specimen data will be placed in the client's existing electronic medical record. All electronic data and files (e.g., database, spreadsheet, etc.) containing identifiable subject information shall be password protected. Any computer hosting such files shall have a BIOS password to prevent access by un-authorized users. If subject data are to be exchanged with others, the data will be coded. If identification is necessary, then the data will be encrypted while en route to the recipient with strong encryption levels (≥ 128 bits for symmetric encryption (DES) and ≥ 1024 bits for asymmetric encryption (RSA).

All data and blood specimens will be stored without direct identifiable information, but will be identifiable via a linking code. The secured research records are labeled with code numbers only (names and other identifying information are kept separate from research records). Access to data is only given to staff members working on the study. Only staff members designated to handle or analyze study samples will have access to the samples and their storage. Coded blood samples are stored in clinic-specific refrigerators and freezers, which are located in secure rooms. As per routine in the UPenn Center for Studies of Addiction (CSA), all electronic files (e.g., database, spreadsheet) will be password protected. Any computer hosting such files will have a BIOS password to prevent access by un-authorized users. Further information concerning the CSA's Data Management Unit (DMU) is provided below.

Blood will be collected for DNA analysis. The information derived from analysis of the subjects' DNA will not be provided to the subject, since at the present time the existing preliminary genetic data for predicting response to ondansetron do not provide a basis for genetic counseling. Should that situation change over the course of the study, procedures will

be developed in conjunction with the UPenn and UMB IRBs to provide subjects with relevant information on their genotypes and to counsel them in relation to that information. While the study is open, DNA samples will be coded with a number that provides an indirect link to the subject's identity (samples will be accessible only by the researchers and staff involved with this study). Upon completion of the study, the sample will be kept in storage indefinitely. However, the sample will forever be separated from all identifiers. These de-identified samples may be shared with other researchers and used in other projects. The lab procedures for storage include a passcode-protected locked room, and secure storage freezers. All samples will be retained securely as per lab protocols.

A certificate of confidentiality has been obtained for this study. The following conditions apply to the protection provided under this certificate:

(l) This certificate does not authorize the University of Maryland, Baltimore, School of Medicine, their contractors or cooperating agencies to refuse to reveal identifying information concerning research subjects if any of the following conditions exist:

(a) The research subject (or, if he or she is legally incompetent, his or her guardian) consents in writing to disclosure of the subject's own identifying information;

(b) Authorized personnel of the United States Department of Health and Human Services request such information for an audit or program evaluation of the research project, or for an investigation of the University of Maryland, Baltimore, School of Medicine, or their contractors or cooperating agencies in carrying out the research project;

(c) Release is required by the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.) or regulations promulgated thereunder (Title 21, Code of Federal Regulations).

(2) This certificate is issued with the expectation that there will be no disclosures of identifying characteristics of research subjects in any Federal, State, or local civil, criminal, administrative, legislative, or other proceedings to compel disclosure of the identifying characteristics of research subjects, except as provided for in paragraph (1), above.

(3) The confidentiality certificate does not govern the voluntary disclosure of identifying characteristics of research subjects, but only protects subjects from compelled disclosure of identifying characteristics. Sponsors/investigators are therefore not prevented from the voluntary disclosure of such matters as child abuse or a subject's threatened violence to self or others; however, the consent form should indicate clearly the investigators' intention to make any such voluntary disclosures.

(4) This certificate does not represent an endorsement of the research project by the Department of Health and Human Services, or the Food and Drug Administration.

(5) All research subjects in the project will be given a fair, clear explanation of the protection which this certificate affords, and of the limitations and exceptions to the protection.

(6) This authority granted to the sponsors/investigators by this certificate is effective upon issuance, and will expire on the date on which the IND application is terminated or withdrawn, or sooner if the sponsor/investigator is notified of cancellation by the Food and Drug Administration. However, regardless of any subsequent expiration or cancellation of the sponsors/investigators' authority under this certificate of confidentiality, the privacy protection afforded to the individual subjects by this certificate of confidentiality is permanent (including after the death of a subject) and will continue for all persons who were research subjects in the study during any time the sponsor/investigator held this certificate of confidentiality.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the IDS, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study will use the web-based direct entry data management system of the CSA's DMU, with data stored on secure servers located in the Perelman School of Medicine's Data Center. Interview data and self-report data from both UPenn and UMB will be entered directly onto computers by research staff and/or patients under supervision by staff. A range of checks for the validity of responses (including field validation to ensure that no out-of-range or otherwise invalid responses are accepted), and form validation to ensure that logically impossible responses to different questions are not accepted) are built into this entry process. After entry, the research staff performs a brief review to ensure that the form has been completed. On completion of this review, the technician will transmit the data (in 128-bit encrypted form) over the Internet to the DMU data servers. After a series of online reviews, the data are archived on the servers. Protected health information will not be included in these research data. Permission to modify the archived data is limited to designated DMU personnel. Audit logs record any modification to the original entry. Various levels of password protection, determined by the UPenn PI, Dr. Kranzler, will allow different members of the research team access to the data. Once the dataset is complete, it will be exported to the biostatistical group for use in modeling.

9.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no

pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan described below. The Principal Investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g., diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

The Principal Investigator and research staff will monitor the study. Written monitoring procedures for monitoring clinical investigations will be implemented to assure the quality of the study and to assure that each person involved in the monitoring process carries out his or her duties.

The Principal Investigator will maintain a record of the findings, conclusions, and action taken to correct errors noted by the monitor for each visit.

Monitoring Responsibilities: The monitor, in accordance with local and NIH requirements, should ensure that the study is conducted and documented properly by carrying out the following activities:

- Verifying that the investigator has adequate qualification and resources and that these remain adequate throughout the study period, and that the staff and facilities, including laboratories and equipment, are adequate to safely and properly conduct the study and these remain adequate throughout the study period.
- Verifying that the investigator follows the approved protocol and all approved amendment(s), if any.
- Verifying that written informed consent was obtained before each subject's participation in the study.
- Verifying that the investigator is enrolling only eligible subjects.
- Reporting the subject recruitment rate.
- Verifying that source data/documents and other study records are accurate, complete, kept up to date, and maintained.
- Verifying that the investigator provides all the required reports, notifications, applications, and submissions, and that these documents are accurate, complete, timely, legible, dated and identify the study.
- Checking the accuracy and completeness of the CRF entries, source data/documents, and other study related records against each other.
- Informing the investigator of any CRF entry error, omission, or illegibility. The monitor should ensure that appropriate corrections, additions, or deletions are made, dated, explained, and initialed by the investigator or by a member of the study staff who is authorized to initial CRF changes for the investigator. This authorization should be documented.

- Determining whether all adverse events (AE's) are appropriately reported within the time periods required by GCP, the protocol, the IRB/IEC, the applicable regulatory requirement(s).
- Determining whether the investigator is maintaining the essential documents.
- Communicating deviations from the protocol, SOP's, GCP, and the applicable regulatory requirements to the investigator and taking appropriate action designed to prevent recurrence of the detected deviations.
- Following the University's SOP's, GCP, and the applicable regulatory requirements.

Study Monitoring
The study Principal Investigator and research staff have prepared a case record form (CRF) that is designed to reduce coding errors and promote data quality. Each page of the CRF will contain a header that includes the title of the protocol, the protocol number, the subject randomization number, subject screening number, and visit number. The technicians are responsible for data entry. The research charts will be maintained separately from the CRF and will contain subject information that is not entered in the study CRF database.

Regulatory Binder: A detailed Regulatory Binder will be assembled. Any changes to procedures are documented in this section. This binder is the backbone of quality assurance. We use this binder to train any new study personnel and for reference so that our policies and procedures are standard throughout all phases of the protocol. The 'Measures' section of the binder addresses, specifically all forms and instruments that comprise the CRF. A Table of Contents has been prepared that contains all the forms used in the study.

The study manual will include:

- study protocol research team names, titles, roles and responsibilities, work and home phone numbers
- purpose of study, study intent and rationale
- all study procedures for each team member (PIs, technicians, study coordinator, pharmacists, nurses, nurse practitioner, physicians, etc.)
- recruitment and screening procedures
- intake procedures
- study phase procedures
- completion/discontinuation procedures

Data Collection Training
The CNC provides comprehensive data collection training for the study staff. Study staff receive training on how to proceed with problematic subjects. Study staff is trained to follow the general data collection guidelines listed below.

- Study staff should be present when subject is completing instruments.
- Research interviews and evaluations should be administered in a private, quiet office or area.
- Instruments are introduced and explained the same way each time to each subject.
- Order of research assessments should be maintained, as much as possible.
- Order of specimens obtained should be maintained as much as possible.
- Study staff should show an interested, polite, appropriate, and helpful demeanor.

Quality assurance (QA) procedures

Changes to the CRF and the study database are strictly monitored. After a single line is drawn through the corrected data point, the study staff documents the change by placing his or her initials and the date of the change adjacent to the correction. Typical QA responsibilities of the study staff include:

- Checking all data during or immediately after study visits.
- Checking charts at predetermined points during the study (initials and date of checker are required on each form checked).
- Performing the initial data check after a few charts have been entered.
- Performing a 10% data check when all data have been entered.

Typical responsibilities of the PI include:

- Monitoring and reporting on study staff interviewing and data collection proficiency at subject study visits.
- Checking charts at predetermined points during the study (initials and date of PI are required on each form checked).
- Supervising study database checks.
- When databases, in Filemaker Pro or other applications, are developed by the research staff, only the data checked by the study staff are entered. Data sets are double checked in pairs by UPENN technicians. The PI supervises any corrections to the data set and reviews the completed database before the information is used in any reports or analyses.
- The PI holds meetings with the study staff to report and review the project and data entry status.
- PI reviews lab results and screening information for potential subjects.
- PI reviews adverse events on at least a weekly basis.

Study Logs and Reports

Study logs are routinely maintained in order to keep computerized back up records of identifying and socio-demographic characteristics and experimental group status. The study subject log usually includes subjects' initials, study number, CRF number, date of birth, race, medication start dates, subject status (active, drop-out, completer, follow-up) and relevant dates and comments.

Data Storage on Site

The CRFs and research charts are stored in file rooms specifically designed for data storage. All study data are housed in locked file cabinets. Only designated members of the research team have access to the file cabinets containing research data. Data are kept on site for not less than three years after the subject has completed the final assessment. Data are then eligible for archiving.

The Principal Investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, and University compliance and quality assurance groups of all study-related documents (e.g., source documents, regulatory documents, data collection instruments, study data). The investigator will ensure that inspections can be made of applicable study-related facilities (e.g., Data Management Unit).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

This study is funded by a grant from the US National Institute on Alcohol Abuse and Alcoholism.

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of Pennsylvania investigators will follow the University conflict of interest policy.

12.3 Subject Stipends or Payments

Subjects will receive payments of $25 for each screening visit(s) and baseline visit(s), $20 each for Visits 1-9 and $50 for the last treatment visit (Visit 10), $10 for returning all medication bottles at each visit ($100 for all 10 medication visits), and $25 if you complete the follow-up visit. Subjects who can not complete the follow-up visit in person can do the assessments by telephone, for which they will be paid $10 when they come to the study clinic. A $100 completion bonus will be given to the participant if he/she completes all study visits and returns all medication bottles. The total amount that subjects can receive for full participation in the research study is up to $555.00. Subjects will receive payment prorated on the basis of the number of visits completed and for incomplete visits, the portion of the visit completed. If a participant does not have transportation to come to a study visit, we can provide transportation at no cost; for example a taxi, bus pass, or providing a car and driver hired through a UMB-approved vendor. If a study visit extends over a mealtime, we will provide them with a meal or snacks at no cost.

Ondansetron Study Participants Payments

Study Visit	Study Week	Payment for Visit	Payment for medication's bottles	TOTAL MAX
V 0-1 Screen (SV)		$ 25 for each visit	0	Up to $ 50
V 0-2 Baseline (BL)	0	$ 25 for each	0	Up to $ 50
V 1	1	$ 20	$ 10	$ 30
V 2	2	$ 20	$ 10	$ 30
V 3	3	$ 20	$ 10	$ 30
V 4	4	$ 20	$ 10	$ 30
V 5	5	$ 20	$ 10	$ 30
V 6	6	$ 20	$ 10	$ 30
V 7	8	$ 20	$ 10	$ 30
V 8	10	$ 20	$ 10	$ 30
V 9	12	$ 20	$ 10	$ 30
V 10	16	$ 50	$ 10	$ 60
V 11	20	$ 10 – by phone $ 25 – in person (with blood draw) $ 100 – completion bonus		
TOTAL		***If V11 by phone $ 10*** + $ 280 (or $305 if 2 SV or $330 if 2 SV and 2 BL) = **$ 290** (or **$315** if 2 SV or **$340** if 2 BL) ***If V11 in person $ 25*** + $ 280 (or $305 if 2 SV or 330 if 2 SV and 2 BL) = **$ 305** (or **$330** if 2 SV or **$355** if 2 SV and 2 BL) ***$ 100 bonus*** – if all study visits completed and all med. bottles returned	MAX **$ 100**	MAX payment UP to: $ 355 + $ 100 + $ 100 = **$ 555**

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