Study Title: Pharmacogenomics and Pharmacometabolomics of Acamprosate Treatment Outcome

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General Study Information

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Research Question and Aims

Introduction
Alcohol Use Disorders (AUDs) are prevalent in our country. The economic and health consequences of AUDs call for efficient treatment strategies. AUDs are difficult to treat, and relapse rates are high, with an estimated 80% of individuals with AUDs returning to alcohol use after completing addictions treatment. Novel treatment approaches are needed to enhance long term sobriety. Our research team has been investigating the use of acamprosate to prevent relapse to alcohol use. Unfortunately despite being FDA approved and endorsed by the American Psychiatric Association only 10% of patients treated for AUD are prescribed acamprosate or other antidipsotropic medications. The number is higher for patients treated in programs affiliated with Mayo Clinic Addiction Services (approximately 20%) but is way less than expected. The most common reasons behind these low numbers are the understanding that not every patient benefits from the use of specific medication and the lack of biomarkers predictive of response. The purpose of this project is to identify such biomarkers by discovery of genomic and metabolomic markers associated with response to acamprosate treatment. To achieve this goal, we will first search for these biomarkers by re-examining findings from several completed AUD treatment studies involving approximately 1,500 patients; next, we will perform a new prospective randomized placebo-controlled trial of 3 month long treatment with acamprosate and search for genetic and metabolomic biomarkers associated with sobriety or relapse in participants of this new study. The combined results of these efforts will provide the largest available study sample for discovery of pharmacogenomic and pharmacometabolomic markers of treatment response in patients with AUD. The discovery of biomarkers of treatment response is expected to improve treatment outcomes by allowing for the personalization of treatment selection.

Specific Aim1: To identify genetic and metabolomic biomarkers associated with acamprosate response in previously collected samples.

Specific Aim1a:
This aim will use existing data from three prior studies [i.e., The Combined Pharmaco-therapies and Behavioral Interventions conducted in the United States (COMBINE Study)[1], a double-blind, placebo-controlled randomized trial conducted in Germany (PREDICT Study)[2], and “A Pharmacogenomic Probe Study of Acamprosate: Genes Associated with Response” Mayo Clinic Center for the Individualized Treatment of Alcohol

study (CITA); total N=1687], to assess the heritability explained by common single nucleotide polymorphisms (SNPs) and the genetic architecture for different measures of alcoholism treatment response. We will use this data to estimate the heritability of different outcomes in patients treated with acamprosate, placebo and naltrexone (as active drug control) that can be explained by common SNPs. We will also investigate the sources of genetic contribution to different treatment outcomes in terms of the distribution of the genetic signal across minor allele frequencies and functional annotation categories. These analyses may help determine if sobriety or another treatment outcome (e.g. return to heavy drinking) is the most suitable phenotype for subsequent pharmacogenomic analyses of acamprosate treatment response.

Specific Aim1b:
This aim will use clinical outcomes data from a previously recruited CITA cohort of 445 acamprosate-treated alcoholic patients and metabolomics data obtained with two metabolomics analytical platforms to identify metabolomic markers associated with length of sobriety as well as additional secondary phenotypes identified in Aim 1a. We will then apply a "pharmacometabolomics-informed pharmacogenomics" research strategy in which we will perform genome-wide association studies in search for genetic variants associated with metabolites associated with acamprosate clinical outcomes. We will pursue all signals, genes and pathways functionally and mechanistically in order to investigate the biological mechanisms underlying sobriety length and other phenotypes associated with acamprosate treatment response. This is the same approach that we used previously with success to study biological mechanisms contributing to effects of selective serotonin reuptake inhibitors in patients with major depressive disorder (MDD).

Specific Aim 2: To identify genetic and metabolomic biomarkers associated with acamprosate response in the new cohort of 800 alcoholic patients randomized to acamprosate or placebo treatment.

Specific Aim 2a:
We will conduct a genome-wide association study (GWAS) in the newly enrolled sample of 800 alcoholic patients. Using acamprosate and placebo arms in the new study cohort will allow identification of genetic markers associated with acamprosate-specific effects impacting treatment outcomes, including sobriety (primary outcome) and other outcomes selected based on the results of Aim 1a. We will also conduct a meta-analysis including existing data from aim 1, which will be the largest pharmacogenomic study of AUD treatment outcomes to date (total N > 2400).

Specific Aim 2b:
To identify metabolomic markers associated with length of sobriety and other acamprosate related clinical outcomes in patients with AUD recruited to the new trial using the same two metabolomic platforms used in Aim 1b in order to replicate and extend results found in Specific Aim 1b and to identify new candidate metabolites. We will then once again apply a "pharmacometabolomics-informed pharmacogenomics" research strategy to pursue the metabolites found to be associated with drug response outcomes during the placebo-controlled study. Similarly to Aim 1b, we will pursue all signals, genes and pathways functionally and mechanistically.

Specific Aim 3:
To apply **a systematic multiple-omics research strategy** to identify molecular and genomic signatures for all AUD samples mentioned in aims 1 & 2, as well as mechanisms underlying individual variation in response to acamprosate. To achieve this goal, we will obtain transcriptomic profiling and proteomic profiling for AUD subjects before and after 3 months of acamprosate therapy. We will then integrate the multiple omics datasets—

i.e., genomics, metabolomics, transcriptomics and proteomics—using machine learning approaches to develop a predictive algorithm for acamprosate response in patients with AUD.

Purpose/Background:
The staggering costs of alcohol use disorders (AUD) call for the development and implementation of evidence-based treatment strategies [3, 4]. The results of the National Survey on Drug Use and Health indicate that less than 10% of those in need consider treatment for alcohol use disorders (Data Spotlight http://oas.samhsa.gov). Moreover, less than 20% of treatment participants remain sober one year later with most of relapses happening during the first 3 months after treatment. The introduction of antidipsotropics (acamprosate and naltrexone) as well as the alcohol deterrent disulfiram has improved abstinence-related outcomes in some, but not all subjects are using those medications (Bouza, Angeles et al. 2004, Suh, Pettinati et al. 2006, Rosner, Leucht et al. 2008). However, only 10% choose to use medication as part of their treatment. It is believed that limited use of these medications is associated with their variable effectiveness, which may be limited to sub-populations of subjects with AUD (Heilig and Egli 2006, Mann, Lemenager et al. 2013). Attention has been called to identification of predictors of pharmacological treatment outcomes in different subsets of alcoholic patients (Addolorato, Mirijello et al. 2013). Yet, attempts to predict efficacy using clinical variables have not been successful (Verheul, Lehert et al. 2005). It is believed that pharmacogenomic research will aid the discovery of such predictors (Kranzler and Edenberg, 2010; Litten et al., 2010).

Treatment selection for subjects with AUD also needs to be considered in the context of frequent comorbidity with other psychiatric disorders and medical conditions. Of those, the most common and clinically significant are depression (Petriks, Gonzalez et al. 2002, Conner, Pinquart et al. 2009) and impaired liver function (Warren and Murray 2013). Findings favor acamprosate for the treatment of alcoholics with depression (Lejoyeux and Lehert 2011), and liver problems (Witkiewitz, Saville et al. 2012), which are common among patients with AUD. On the contrary, naltrexone and disulfiram carry potential risk for liver toxicity (Krampe and Ehrenreich 2010, Achunine and Taylor 2012). Acamprosate is also the most widely used medication for AUD treatment (Mark, Kassed et al. 2009) and the proper determination of potential responders and non-responders may result in considerable savings in treatment costs (Schadlich and Brecht 1998, Annemans, Vanoverbeke et al. 2000, Mason and Crean 2007). Therefore, the identification of biomarkers predicting the ability to respond to acamprosate treatment will be a major public health benefit in terms of identifying AUD patients likely to achieve a positive outcome and the least side-effects (Mason and Heyser 2010, Hyman 2014).

The FDA approved acamprosate, naltrexone and disulfiram as adjuncts to psychosocial treatment of AUD. Consistently, the NIAAA website recommends use of those along with behavioral treatment and mutual support groups, emphasizing that “there is no one-size-fits-all solution” (https://pubs.niaaa.nih.gov/publications/Treatment/treatment.htm#chapter02). Similarly, contemporary American Psychiatric Association (APA) Guidelines recommend that naltrexone or acamprosate be offered to patients with moderate to severe alcohol use disorder who have a goal of reducing alcohol consumption or achieving abstinence, prefer pharmacotherapy or have not responded to nonpharmacological treatments alone and have no contraindications to the use of these medications (https://psychiatryonline.org/doi/pdf/10.1176/appi.books.9781615371969).

Consistent with the above mentioned guidelines, all patients treated in the Intensive Addictions program affiliated with Mayo Clinic Addiction Services are being offered FDA approved medications for AUD treatment. The physician discusses the benefits and risks of adding antidipsotropic medications in the context of individualized

assessment of patient's treatment needs. In our experience only 15-20% of patients choose to add acamprosate to their treatment.

Clearly, response predictors are needed to improve treatment and it is expected that pharmacogenomic research will aid in the discovery of such predictors [7, 8]. Moreover, the discovery of biological mechanisms of response to acamprosate as a "probe drug," may guide development of innovative treatment approaches and personalized recommendations for the treatment of patients with AUD.

We previously demonstrated an association of the glutamate ionotropic receptor N-methyl-D-aspartate (NMDA) type subunit 2B *(GRIN2B)* rs2058878 variant with sobriety length in two independent samples of acamprosate-treated alcoholics [9]. Yet, these findings were based on a candidate gene/pathway approach and potentially missed other important variations, suggesting a need for an unbiased and comprehensive search for genetic markers of response. Moreover, although clinical data favors sobriety as a treatment outcome associated with acamprosate, no studies have explored whether sobriety is more heritable than other alcoholism treatment outcomes, and thus it is unknown whether sobriety is the most appropriate outcome for pharmacogenomics investigations. Furthermore, the intermediate phenotypes, which are more proximal to genetic variation compared to behavioral phenotypes, are known to improve the power of genetic association studies [10, 11]. We have previously demonstrated that change in the plasma metabolite levels is a powerful intermediate phenotype allowing for the identification of genes associated with treatment response to selective serotonin reuptake inhibitor (SSRI) antidepressants – an approach called 'pharmacometabolomics-informed pharmacogenomics' [12]. However, this powerful approach has not been used in pharmacogenomic studies of antidipsotropic medications. Yet, preliminary metabolomic analyses of our data indicated that acamprosate responders had elevated serum glutamate at baseline, which decreased during treatment [13]. Similarly, our brain imaging studies showed an association of glutamate levels in the left dorsolateral prefrontal cortex with alcohol cravings and response to acamprosate treatment [14, 15].

Thus, our preliminary findings indicate that genetic variation as well as metabolite (glutamate) levels in human plasma and brain tissue may be associated with acamprosate response. Yet, these findings, based on a candidate gene/pathway approach, potentially ignored other important variations and the lack of placebo arm in the original study precluded the differentiation between acamprosate-specific associations from those related to other factors contributing to sobriety. Moreover, we have only investigated genetic associations with sobriety and it is unknown if stronger association may exist with other treatment outcomes. Therefore, the goal of this study is to identify genetic markers associated with acamprosate response by using highly innovative strategies including: (1) polygenic analysis of existing data from genome-wide studies of acamprosate response to determine appropriate phenotype for pharmacogenetic study and (2) search for genetic markers associated with acamprosate vs. placebo treatment response in AUD patients on a genome-wide scale in the combined sample including alcoholics treated by acamprosate and placebo in the COMBINE, PREDICT and CITA studies and a new sample of 800 AUD patients treated in community-based programs in a double blind randomized placebo controlled study of acamprosate. This will allow us to perform a meta-analyses of genome-wide meta-analyses of AUD treatment outcomes in the largest combined sample used for pharmacogenomic studies in the field of alcoholism research (total N>2400). This project involves an innovative, integrated and comprehensive series of studies of the pharmacometabolomics and "pharmacometabolomics-informed pharmacogenomics" of acamprosate in the treatment of patients suffering from AUD. The experiments proposed subsequently have been designed to identify metabolomic biomarkers for response to acamprosate therapy and then to subject those metabolomic biomarkers to genome-wide association studies (GWAS) to identify SNPs and genes associated with concentrations of the metabolites associated with drug response phenotypes—SNPs and genes which can then be

pursued functionally for their biological plausibility and to gain insight into novel mechanisms underlying the observed associations.

Study Design and Methods

Research Design

Specific Aim1: To identify genetic and metabolomic biomarkers associated with acamprosate response in previously collected samples.

Specific Aim1a:
Statistical analyses will be applied to assess the heritability explained by common single nucleotide polymorphisms (SNPs) and the genetic architecture of different measures of outcomes of treatment with acamprosate, placebo and naltrexone (as active drug control). We will also investigate the sources of genetic contribution to different treatment outcomes in terms of the distribution of the genetic signal across minor allele frequencies and functional annotation categories using advanced statistical analysis methods as described under analysis plan. These analyses may help determine if sobriety or another treatment outcome (e.g. return to heavy drinking) is the most suitable phenotype for subsequent pharmacogenomic analyses of acamprosate response.

Specific Aim1b:
We will apply a "pharmacometabolomics-informed pharmacogenomics" research strategy in which we will identify metabolomic markers associated with length of sobriety as well as additional secondary phenotypes followed by performing genome-wide association studies for the metabolites associated with acamprosate clinical outcomes. We will then pursue all signals, genes and pathways functionally and mechanistically.

Specific Aim 2: To identify genetic and metabolomic biomarkers associated with acamprosate response in the new cohort of 800 alcoholic patients randomized to acamprosate or placebo treatment.

Specific Aim 2a:
We will conduct a double-blind randomized placebo-controlled study of treatment response to acamprosate in the newly enrolled sample of 800 patients with AUDs participating in the community based treatment programs and use clinical outcomes of this study for genome-wide association analyses searching for genetic markers associated with treatment outcomes.

Specific Aim 2b:
To identify metabolomic markers associated with length of sobriety and other acamprosate related clinical outcomes in 800 patients with AUD recruited to a new trial in which the patients will be randomized to acamprosate or placebo using the same two metabolomic platforms used in Aim 1b in order to replicate and extend results found during the earlier non-placebo controlled study of 445 AUD patients and to identify new candidate metabolites. We will then apply a "pharmacometabolomics-informed pharmacogenomics" research strategy to pursue the metabolites found to be associated with drug response outcomes during the placebo-controlled study.

Using acamprosate and placebo arms in the new study cohort will allow identification of genetic markers associated with acamprosate-specific effects impacting treatment outcomes, including sobriety (primary outcome) and other outcomes selected based on the results of Aim 1. We will also conduct a meta-analysis of

pharmacogenomic effects on acamprosate response, which will be the largest pharmacogenomic study of AUD treatment outcomes to date (total N > 2000).

To accomplish Aims 2a and 2b, 800 patients meeting Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM5) criteria for AUD will be recruited from community-based treatment programs affiliated with Mayo Clinic and Mayo Health System and Hazelden Betty Ford Foundation and enrolled in a double-blind randomized placebo-controlled trial of acamprosate (Fig. 1).

Specific aim 3:
To accomplish Aim 3, we will be collecting and storing additional blood samples for multiple-omics methods to identify genetic and other markers associated with acamprosate treatment outcomes.. For example, state-of-the-art induced pluripotent stem cells (iPSC) technology has undoubtedly become a powerful research tool which holds great potential in neuropsychiatric research, including addiction research. The iPSCs offer a unique opportunity to create cell types (both normal and pathological phenotypes) for target tissues from a variety of organs, for example but not limited to, the central nervous system, the brain, and "organoids". Due to the high cost and the lengthy process required for iPSC generation, we will generate a panels of iPSCs for functional genomics studies. These genomic studies for all AUD samples mentioned in aims 1 & 2, when combined with metabolomic, proteomic, transcriptomic, and other molecular studies, and clinical outcomes will provide an opportunity to apply multiple "omic" approaches to obtain novel insight into the underlying pathophysiology of AUD, which will serve as a unique scientific resource.

Recruitment process and infrastructure
Recruitment sites: Patients will be recruited from study sites affiliated with the Mayo Clinic Addiction Services in the Rochester and Albert Lea campuses (Intensive Addiction treatment Program, Fountain Center Residential Treatment Program in Albert Lea, MN, Department of Psychiatry & Psychology Mayo Clinic Albert Lea) and Hazelden Betty Ford Foundation treatment Centers in Center City, MN and Plymouth, MN. Hazelden Betty Ford Foundation St. Paul location will be an alternative site to conduct study visits (no recruitment activities) to improve options for patient traveling while participating. With the integration of Rochester and the Health System sites into Mayo Midwest Department of Psychiatry & Psychology we have the ability to recruit at these campuses in the context of the integration and standardization of electronic medical records, nomenclature, clinical practices, and optimization of data capture, management and reporting. In addition, arrangements were made with the leadership of Hazelden Betty Ford Foundation allowing ensuring efficient enrollment process and data collection at the affiliated study sites. A brief description of each treatment program is presented below. Recruitment sites of Mayo Health System Albert Lea and Hazelden Betty Ford Foundation Treatment Centers (Center City & Plymouth) will rely on Mayo Clinic IRB and will be added when agreements are in place.

1. The Department of Psychiatry and Psychology at the Mayo Clinic in Rochester, MN has two treatment programs for persons 18 and older, which integrate a combination of psychosocial treatments with evidence-based pharmacotherapies of addiction under the direction of Board Certified addiction psychiatrists. The Intensive Addiction Program (IAP) is a 30-day residential treatment program that provides treatment 9 hours per day, 6.5 days per week, and the Outpatient Addiction Program (OAP) that provides an outpatient treatment program limited to 4 hours a day, 5 days per week. The combined annual census of the IAP and the OAP is 267 patients.

2. The Fountain Center Residential Treatment Program in Albert Lea, MN is a counselor-lead residential program for persons 18 and older with an emphasis on teaching relapse prevention skills. Fountain Center integrates the 12 Steps of Alcoholics Anonymous into the program. Annual census is 201 patients. Mayo has recently provided a shuttle service between Rochester and Albert Lea.

3. The Hazelden Betty Ford treatment facility in Center City, MN is a 140 bed residential, and outpatient treatment facility which serves persons 18 and older with Alcohol and Substance Use Disorders. The goal of the program is to ensure each client's health returns to daily living through intensive, comprehensive treatment. Annual census is ~1500 patients including ~80% with AUD.

4. The Hazelden Betty Ford treatment facility in Plymouth, MN is a residential and outpatient treatment facility with specialized programming for adolescents, teenagers, and young adults ages 12-25 with alcohol and substance use disorders. Annual census is ~ 815 patients.

5. The Hazelden Betty Ford treatment facility in Newburg, OR is a residential and outpatient treatment facility which serves persons 18 and older with alcohol and substance use disorders. Annual census is ~ 850 patients.

Study Assessments and Procedures

Assessments

A standardized clinical assessment is performed for all patients admitted to above mentioned treatment programs. This assessment will be used by study personnel to determine study eligibility, and determine if there is a need to discuss treatment of depression and/or anxiety. The Study Psychiatrist affiliated with each study site, or a member of the clinical treating team will meet with the patient to assess appropriateness and discuss potential benefits and risks associated. For patients meeting study inclusion and exclusion criteria and signing Informed Consent, the presence of AUD and comorbid conditions including depressive and anxiety disorders will be confirmed by Psychiatric Research Interview for Substance and Mental disorders (PRISM). The severity of depression and anxiety symptoms will be assessed by the Patient Health Questionnaire (PHQ-9) and Generalized Anxiety Disorder (GAD-7), respectively. In addition, alcohol use history, alcohol craving intensity, LFT levels and other relevant measures will be assessed at baseline and repeated at follow up visits as defined in Table 1.

(1) We will standardize depression and anxiety treatment by accepting current and stable use of SSRI & SNRI medications listed in table 3 (page 14) in all participants with depression and/or anxiety diagnoses. This will allow for an assessment of the impact of depression and/or anxiety as well as their standardized treatment on acamprosate and placebo-related treatment outcomes and account for this impact in genetic and metabolomic association analyses. Patients with newly diagnosed anxiety or depression as well as patients with known diagnosis but not taking any antidepressant will be offered treatment with escitalopram or citalopram as first line treatment during study participation when their GAD-7 or PHQ-9 scores are equal or above 10. We selected escitalopram based on evidence suggesting it is strongest among selective serotonin reuptake inhibitors (SSRIs) [18] and favoring it over other antidepressants in terms of efficacy and tolerability [19]. Clinical findings in adults with AUD and depression as well as our experimental data also suggest that the combined use of escitalopram and acamprosate may improve treatment outcomes [20, 21]. This threshold has been chosen based on previous research showing that PHQ-9 score ≥ 10, indicative of the presence of at least moderate depression and/or a GAD-7 score of ≥ 10, indicative of the presence of at least moderate anxiety reflect a clinically significant level of symptom severity warranting the use of medications [16, 17]. Results of treatment with escitalopram or citalopram will be monitored by the study

psychiatrist affiliated with each study site who will make appropriate treatment recommendations as described below.

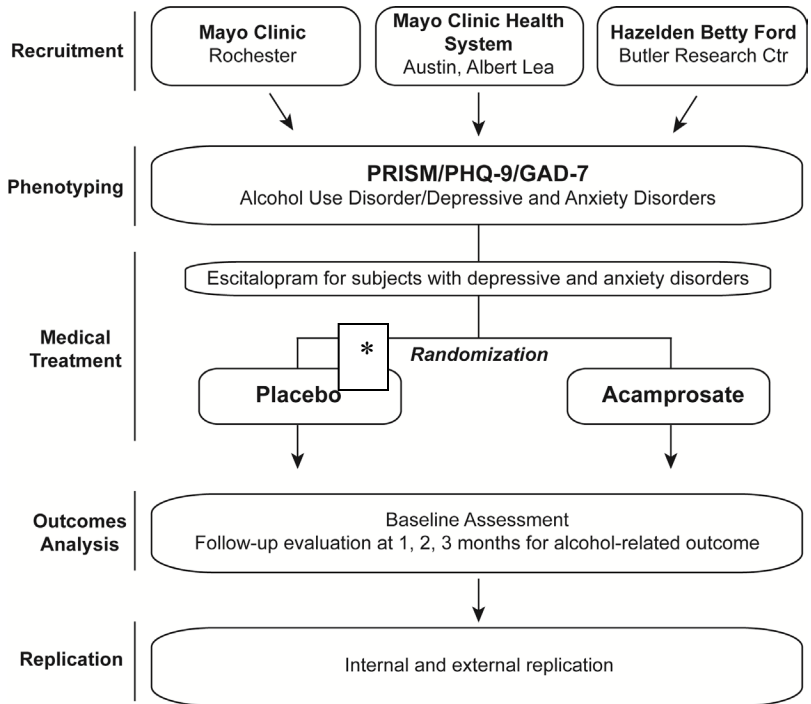
(2) To avoid the impact of end stage liver disease on clinical and metabolomic findings, we will use a Model for End-Stage Liver Disease (MELD) score of 10 and below as a cut off for *inclusion* in the study, and will *exclude* patients with a diagnosis of advanced liver disease or those with a MELD score above 10, which is considered an indication that the extent of liver damage has reached an advanced stage [22, 23]. The MELD score will be calculated according to the standard approach using serum concentrations of bilirubin and creatinine, and the international normalized ratio for prothrombin time [22].

Study flow and Schedule of Events

Study personnel will explain the research risks and benefits. Written informed consent will be obtained for each study subject. The baseline diagnostic assessment, triage, treatment group assignment and follow up assessments are summarized in Fig. 1 and described in more detail in Specific Aim 2. Information regarding severity of alcohol withdrawal symptoms (measured by CIWA scale or site specific equivalent method) and medications used during withdrawal treatment preceding enrollment in the study will be collected or abstracted from the medical record and included in the data analyses.

*Notation dated 5-12-2023: Upon IRB approval, subjects will no longer be randomized to the placebo arm. Instead, for the remainder of the study, all patients will be provided acamprosate to achieve the active drug and placebo ratio of 2:1.

Figure 1: Study Flow Chart



The presence of AUD and other psychiatric disorders will be confirmed by a computer version of the Psychiatric Research Interview for Substance and Mental Disorders (PRISM) [24]. The presence of comorbid depressive disorders requiring treatment will be determined by the combination of 2 characteristics: (1) the presence of any

diagnosis of depression during the previous year as confirmed by PRISM and verified by the study psychiatrist; and (2) a PHQ-9 score of 10 or above and/or a GAD-7 score of 10 or above. The interview will be conducted by interviewers trained and certified on the use of the PRISM. Interviewers, who have submitted required recordings and documents for certification and an unexpected delay in the certification process occurs, will be allowed to conduct prism assessment, if no other certified staff are available, with certification pending. The research pharmacy contracted for this study will randomize the study participants for all sites with placebo or acamprosate.

The schedule of study procedures and assessments is tabulated by visit in the Study Assessments in Table 1 (below). A visit window of +/- 10 days is allowed when scheduling. Questionnaires may be distributed in multiple formats (paper, electronic/emailed link, or asked/read to patient.

MAYO CLINIC

Table 1: Study Assessments							
Study Assessments	**Baseline Evaluation**	**Follow-Up Visits**					
		2 week – TC	**1 month ♯**	**6 week – TC**	**2 month ♯**	**10 week – TC**	**3 month ♯**
Blood Collection	**X**		**X**				**X**
Medication provided	**X**		**X**		**X**		
PRISM	**X**						
LTDH	**X**						
CIWA-Ar	**X**						
TLFB ♦ (BDQ)	**X**		**X**		**X**		**X**
PHQ-9	**X**	**X**	**X**	**X**	**X**	**X**	**X**
GAD7	**X**	**X**	**X**	**X**	**X**	**X**	**X**
AAM			**X**		**X**		**X**
IDTS	**X**						
PACS	**X**		**X**				**X**
PRISE	**X**		**X**		**X**		**X**
FIBSER			**X**		**X**		**X**
PSQI	**X**		**X**		**X**		**X**
FIRM	**X**						
CD-RISC	**X**						**X**
Med. Compliance			**X**		**X**		**X**
Med. Compliance Enhancement		**X**		**X**		**X**	
CTA	**X**						
GGT lab test	**X**		**X**				**X**
CTQ	**X**						
CGI			**X****		**X****		

Definitions: AAM: AA attendance monitoring. CIWA-R: Clinical Institute Withdrawal Assessment-Research. CTA: Commitment to Abstinence. CTQ: Childhood Trauma Questionnaire. FIBSER: Frequency, Intensity and Burden of Side Effects Ratings. GGT: gamma-glutamyl transpeptidase lab test. IDTS: Inventory of Drug Taking Situations. LTDH: Lifetime Drinking History. PACS: Penn Alcohol Craving Scale. PHQ-9: Patient Health Questionnaire 9-item. GAD7: Generalized Anxiety Disorder 7-item. PRISE: Patient-rated Inventory of Side Effects. PRISM: Psychiatric Research Interview for Substance and Mental Disorders. TC: Telephone Call TLFB: The Alcohol Timeline Follow Back. PSQI: Pittsburgh Sleep Quality Index. FIRM: Family Index of Risk for Mood. CD-RISC: Connor-Davidson Resilience Scale (CD-RISC); CGI: Clinical Global Impression (CGI); **Used by study psychiatrist to assess worsening mood symptoms despite antidepressant treatment during study participation. ♦ Brief Drinking Questionnaire (BDQ). ♯ Allow the use of alternative formats to face-to-face visits based on concern for COVID exposure or other circumstances potentially preventing patients from attending face-to-face visits (blood draw done in person).

Description of Assessment Instruments:

PRISM: Psychiatric Research Interview for Substance and Mental Disorders (PRISM) will be used for standardized assessment of Substance Use Disorders and comorbid conditions. PRISM is an electronic computer-based tool providing systematic coverage of the longitudinal course of alcohol- and drug-related experiences and psychiatric symptoms that may be useful in identifying areas of focus for treatment. The PRISM follows a decision-tree format using diagnostic algorithms for all Axis 1 psychiatric disorders plus Borderline and Antisocial Personality Disorders. Acceptable reliability and diagnostic validity of PRISM has been demonstrated [25, 26].

LTDH: The Lifetime Drinking History questionnaire (LTDH) provides a self-report of patterns of alcohol consumption over a person's lifetime [27]. A modified version allowing for the assessment of initial tolerance and maximum alcohol consumption in a 24-hour period will be used to provide the clinical projects with a description of the study samples and to investigate the potential impact of the LTDH on the primary and secondary treatment outcomes.

CIWA-Ar: The revised Clinical Institute Withdrawal Assessment for Alcohol (CIWA-Ar) scale. This assessment for monitoring withdrawal symptoms requires approximately 5 minutes to administer. The maximum score is 67. Patients scoring less than 10 do not usually need additional medication for withdrawal [28].

TLFB: The Alcohol Timeline Follow Back (TLFB) is a drinking assessment method that obtains estimates of daily drinking and has been evaluated with clinical and nonclinical populations. Using a calendar, people provide retrospective estimates of their daily drinking over a specified time period that can vary up to 12 months from the interview date[29].

PHQ-9: The 9-Item Patient Health Questionnaire (PHQ-9). The PHQ-9 consists of nine questions, rated 0–3 according to the increased frequency of difficulty experienced in each area covered. Scores, with a possible range of 0–27, are summed and can then be interpreted as follows: no depression (0), minimal (1–5), mild (6–9), moderate (10–14), moderately severe (15–19), or severe (>20) depression.

GAD-7: Generalized Anxiety Disorder 7 (GAD-7) is a self-reported questionnaire for screening and severity measuring of generalized anxiety disorder (GAD). GAD-7 has seven items, which measure severity of various signs of GAD according to reported response categories with assigned points. This will take approximately 5 minutes to complete.

AA Monitoring: Self-monitoring of attendance of Alcoholics Anonymous meetings (AAM) and sponsorship [30] will be used as a potential covariate and predictor of treatment response.
Evidence supports the importance of motivation for sobriety and attendance of support groups for treatment outcomes in alcoholics [31, 32]. Therefore, we will collect CTA and AAM data to allow for the assessment of the impact of these factors on treatment outcomes.

IDTS: The Inventory of Drug-Taking Situations (IDTS), developed by Annis and Martin (1985) [33], is a 50-item self-report questionnaire that provides a profile of the situations in which a client has used alcohol or another drug over the past year. Clients are asked to indicate their frequency of heavy drinking or drug use in each of 50 situations on a 4-point scale ranging from "never" to "almost always." The questionnaire may be administered in either pencil-and-paper or computerized version; the latter allows a client to name up to three substances that are currently causing a problem; the 50 IDTS items are presented for each substance in turn, and a computer-generated report is produced for each substance.

PACS: The Penn Alcohol Craving Scale (PACS) is a five-item self-administered instrument for assessing frequency, intensity, and duration of craving [34, 35]. The questions on the PACS use descriptors coupled with numerical ratings ranging from 0 to 6. PACS will be used to assess intensity of craving at baseline and follow up visits as a potential predictor of treatment response.

Intensity of craving and its contextual meaning (e.g. negative or relief craving) were associated with abstinence from alcohol and acamprosate response [36-38]. Therefore, we will collect PACS and IDTS data to allow for assessment of the impact of these factors on treatment outcomes.

PRISE: Patient-Rated Inventory of Side Effects (PRISE) is a self-report form used to qualify adverse effects as tolerable or distressing in 9 different domains, each with multiple symptoms [39].

FIBSER: Frequency, Intensity and Burden of Side Effects Ratings (FIBSER) is a self-report form used to quantify the adverse effect burden [40].

CTA: Commitment to Abstinence (CTA) will be determined by the subject's response to a questionnaire developed by Hall and colleagues in 1990 and modified with permission [41].

CTQ: The Childhood Trauma Questionnaire (CTQ) was developed as screening tools for histories of abut and neglect. The self-report includes a 28-item test that measures 5 types of maltreatment – emotional, physical, and sexual abuse, and emotional and physical neglect. Approximately 5 minutes is required to complete the test. A 5-point Likert scale is used for the responses which range from Never True to Very Often True**.**

FIRM: Family Index of Risk for Mood (FIRM) is a self-reported tool developed to gather family history of psychiatric and seizure illness. The FIRM has demonstrated clinical significance in detecting pediatric bipolar disorder. [44]

CD-RISC: Connor-Davidson Resilience Scale (CD-RISC) is a self-rating scale to assess resilience. Resilience may be viewed as a measure of stress coping ability and, as such, could be an important target of treatment in anxiety, depression, and stress reactions. The CD-RISC has sound psychometric properties and distinguishes between those with greater and lesser resilience. [45]

CGI: Clinical Global Impression (CGI) is an assessment used by study psychiatrist for clinical judgment of depression symptoms' severity and worsening symptoms during the course of study participation. [46]

Side Effects Assessment: Assessment of side effects is critical to ensure safety of study participants and for the assessment of tolerability of the treatments. Therefore, we will collect PRISE and FIBSER data to monitor study participants for safety during treatment with study medications and to allow for the assessment of the impact of these factors on treatment outcomes.

PSQI: Pittsburgh Sleep Quality Index is a self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval. Nineteen individual items generate seven "component" scores including: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction.

BDQ: Brief Drinking Questionnaire is used as an alternative to collecting drinking information if a patient refuses to complete the TLFB assessment. Six questions are asked to assess relapsed drinking during a period of time (i.e. between follow-up visits).

Remuneration:

All participants will be paid $40 each for completing Baseline, Month 1 and Month 2 visits and paid $50 for completing the Month 3 visit for a total of $170. Non-Mayo patients will be given a Visa gift card for the appropriate dollar amount corresponding to completed visits. Mileage reimbursement will be available for travel costs up to the amount of $75 per visit.

Study Medications:
All participants will be randomized to receive acamprosate or placebo* in a double-blinded placebo-controlled trial. Acamprosate, SSRIs, and SNRIs listed are FDA approved medications for the indications for which they are being used in this protocol; therefore the risk to participants is no more than that to the general population who may be taking these medications. Medication/Placebo* administration starts with 1 pill 3 times a day for 7 days then increases to 2 pills 3 times a day.

*Notation dated 5-12-2023: Upon IRB approval, subjects will no longer be randomized to the placebo arm. Instead, for the remainder of the study, all patients will be provided acamprosate to achieve the active drug and placebo ratio of 2:1.

Potential Side Effects: Participants could be at risk of the potential side effects of one or both of these medications, which they will be made aware of at the time of obtaining informed consent and will be monitored on a monthly basis. Acamprosate is reasonably well tolerated in most patients. The most common side effect associated with acamprosate use is diarrhea, which occurs in approximately 16% of patients. Other frequently occurring side effects include asthenia, nausea, pruritus, and flatulence, headache, abdominal pain, flu syndrome, edema, weight gain, and myalgia. Infrequent side effects include ascites, face edema, photosensitivity reaction, suicidal ideation, suicide attempts, completed suicide, and acute kidney failure. The potential serious side effects of acamprosate are rare and include cardiomyopathy, deep vein thrombophlebitis, heart failure, and mesenteric arterial occlusion. The common side effects of SSRIs, and SNRIs listed on table 3 (page 14) are, but not limited to, diaphoresis, abdominal pain, constipation, diarrhea, indigestion, nausea, vomiting, xerostomia, dizziness, headache, insomnia, somnolence, disorder of ejaculation, impotence, orgasm incapacity, reduced libido, and fatigue. The potential serious side effects of are worsening depression, suicidal thoughts, and serotonin syndrome. It is possible that the intensity of depressive or anxiety symptoms may increase despite treatment and patients may develop suicidal ideation. It is also possible that patients may become medically unstable due to conditions unrelated to study treatment. The study team will meet regularly to review any side effects or adverse events reported and modify the protocol accordingly. Study participants developing side effects requiring medical attention will be referred to appropriate medical facilities.

In-study risks and safety procedures:
General considerations: Participant's treatment response as well as frequency and intensity of adverse effects will be discussed at baseline and assessed during follow-up visits with study staff. The study blind will be removed if any Unanticipated Problems Involving Risk To Subjects or Others (UPIRTSO) or adverse events is determined to be potentially related to the study medication (as deemed by treating study psychiatrist and study PI). Should concerns emerge regarding the patient's clinical problems not related to this study, those should be addressed by primary care specialist of patient's choice If deemed necessary by study personnel, an urgent discussion with a study psychiatrist will take place for evaluation, and appropriate referral for treatment will be made. Study consent form includes participant's consent for permission to interface with his/her primary provider should clinical concerns arise.

Depression Safety Management Plan: The study team members will not be providing clinical care for conditions other than treatment of AUD and comorbid depression or anxiety. Therefore, any findings from PRISM assessment raising safety concerns, including positive response to suicide questions will trigger immediate notification to study psychiatrist and patient's clinician. In addition, if a subject endorses suicidality with any of the following: Active suicidal ideation as determined by the investigator or as determined by PHQ-9 (Question 9 score of 1 or higher) or if a subject responds positively to questions regarding the harm of self and/or others, the research team will immediately contact the study psychiatrist to refer for appropriate treatment.

During the follow up visits, questionnaires that involve questions related to depression not involving immediate concerns of harm to self or others will be reviewed within 1 working day of receipt from the subject. Changes reflecting worsening of depressive and anxiety symptom severity scores as collected using PHQ-9 and GAD-7, respectively, will be reported to the study psychiatrist who will determine if further assessment is necessary to ensure appropriate medical care and the safety of the study participant. The Clinical Global Impression (CGI) tool will be used to reflect assessment of those symptom changes by study psychiatrist. Study participants whose condition deteriorates to the point of being judged unstable (defined as a score of ≥ 6 on CGI) by a study psychiatrist, will be withdrawn from the study and referred to appropriate treatment. If emergent concerns arise regarding risk of harm to self or others, the patient will be referred for emergency care; if declined, authorities will be contacted for a well person check. Study PI or covering MD will be available to discuss questions related to management of depression, anxiety or other study-related aspects of patient care.

Resources:
We will utilize substantial resources available at Mayo Clinic, such as the Clinical Research Unit (CRU), to support recruitment and proper specimen collection and procurement at the Mayo Health System sites. The addition of the large clinical operation at the Hazelden Betty Ford Foundation treatment facilities in Center City and Plymouth, MN will significantly increase the referral base allowing for a more rapid achievement of the recruitment targets; and trained personnel (in all research sites) enabling large-scale prescreening, selection, enrollment and monitoring of the study participants will be required to achieve this goal. Adding Hazelden St. Paul location will improve visit options for patient travel while participating in the study.

Genome-wide genotyping will be conducted by the Mayo Clinic 'Medical Genome Facility', which offers a variety of genotyping assays, including state-of-the-art genome-wide SNP arrays.

The amino acid metabolomic platform assays using the CITA samples will be performed at the Mayo Clinic Metabolomic Core Facility. During year 2, metabolomic assays for neuromodulator metabolites will be performed. The acamprosate placebo-controlled trial recruitment will continue throughout years 1, 2 and 3—with completion of the trial at the end of year 3.

The Statistics Team, which will be responsible for data management, biostatistics and bioinformatics support. The statistical team (Dr. Biernacka, Postdoc, MS Statistician, and Statistical Programmer) will meet weekly to plan and review ongoing data analyses. Dr. Joanna Biernacka, the Statistics Team Director and Co-PI for this study, obtained a PhD degree in biostatistics, with a focus on statistical genetics, followed by further training in statistical genetics as a postdoctoral research fellow. She joined Mayo Clinic's Program in Genomics of Addiction ten years ago.

☒ (1a) This is a multisite study involving Mayo Clinic and non Mayo Clinic sites. *When checked, describe in detail the research procedures or activities that will be conducted by Mayo Clinic study staff.*

☐ (1b) Mayo Clinic study staff will be engaged in research activity at a non Mayo Clinic site. *When checked, provide a detailed description of the activity that will be conducted by Mayo Clinic study staff.*

Subject Information

Target accrual: 800 (400 Mayo Clinic; 400 Hazelden Addiction Treatment Centers)

Subject population: Adults (male and female)

Inclusion Criteria:

1. Age 18 to85; DSM-5 (14) diagnosis of AUD determined by PRISM;
2. Completion of alcohol detoxification (CIWA score < 5) and no alcohol for at least 7 days (but no more than 35 days);
3. Ability to provide informed consent
4. Ability to speak English
5. Willingness to use the study medications for 3 months and attend follow-up visits.
6. No chronic/daily use of benzodiazepines, opioids, or stimulants for a period of time which is determined by 3 x the medication half-life value (see addendum A) to be completed before the initiation of study medication (acamprosate or placebo).
7. Willingness to discontinue previously prescribed acamprosate for a period of at least 3 days before randomization to study medication (acamprosate or placebo*) which allows for metabolomic signature without medication.

*Notation dated 5-12-2023: Upon IRB approval, subjects will no longer be randomized to the placebo arm. Instead, for the remainder of the study, all patients will be provided acamprosate to achieve the active drug and placebo ratio of 2:1.

Exclusion Criteria:

1. Hypersensitivity or allergy to acamprosate
2. Current use of wellbutrin and not willing to switch to an acceptable antidepressant medication
3. Renal impairment (creatinine level >1.5 mg/dL);
4. Diagnosis of advanced liver disease indicated in the medical record or by a MELD score of above 10;
5. Women who are pregnant, breastfeeding, or planning to become pregnant during the next year;
6. Primary diagnosis of substance use disorder other than alcohol as determined by PRISM or in medical record review or secondary diagnosis of active (within the past year) benzo/sedative dependence, opioid dependence, stimulant dependence, heroin dependence, and/or cocaine dependence
7. Refusal to abstain from any chronic/daily use of prescribed benzodiazepines, opioids, stimulants, cannabis related medication such as CBD or medical marijuana, during the course of participation.
8. Current use of Naltrexone and not willing to stop and switch to Acamprosate/Placebo*(see notation dated 5-12-2023 above)
9. Current use of Antabuse.

10. Active suicidal ideation or any unstable medical or psychiatric condition as determined by responses to PRISM or by the investigator.
11. Status of involuntary or court-ordered admission at time of consent.

Research Activity

Check all that apply and complete the appropriate sections as instructed.

1. ☐ **Drug & Device**: Drugs for which an investigational new drug application is not required. Device for which (i) an investigational device exemption application is not required; or the medical device is cleared/approved for marketing and being used in accordance with its cleared/approved labeling. (Specify in the Methods section)

2. ☒ **Blood**: Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture.

3. ☐ **Biological specimens other than blood**: Prospective collection of human biological specimens by noninvasive means that may include: urine, sweat, saliva, buccal scraping, oral/anal/vaginal swab, sputum, hair and nail clippings, etc.

4. ☐ **Tests & Procedures:** Collection of data through noninvasive tests and procedures routinely employed in clinical practice that may include: MRI, surface EEG, echo, ultrasound, moderate exercise, muscular strength & flexibility testing, biometrics, cognition testing, eye exam, etc. (Specify in the Methods section)

5. ☒ **Data** (medical record, images, or specimens): Research involving use of existing and/or prospectively collected data.

6. ☐ **Digital Record**: Collection of electronic data from voice, video, digital, or image recording. (Specify in the Methods section)

7. ☐ **Survey, Interview, Focus Group:** Research on individual or group characteristics or behavior, survey, interview, oral history, focus group, program evaluation, etc. (Specify in the Methods section)

☐ NIH has issued a *Certificate of Confidentiality* (COC). *When checked, provide the institution and investigator named on the COC and explain why one was requested.* _____

Biospecimens – Categories 2 and 3

(2) Collection of blood samples. When multiple groups are involved copy and paste the appropriate section below for example repeat section b when drawing blood from children and adults with cancer.

a. **From healthy, non-pregnant, adult subjects who weigh at least 110 pounds**. For a minimal risk application, the amount of blood drawn from these subjects may not exceed 550ml in an 8 week period and collection may not occur more frequently than 2 times per week.

Hazelden Betty Ford Treatment facilities in Plymouth, MN and Newburg, OR, Mayo Clinic Health System site in Albert Lea, MN patient blood volume per blood draw: 35ml

Other location's (Mayo Clinic Rochester & Hazelden Betty Ford, Center City, MN) patients' blood volume per blood draw: 50ml at Baseline (preferred but can be done at any visit) and 35 ml at Months 1 & 3
Hazelden Center City will offer participants to provide the additional ~15ml of blood as optional (preferred at baseline visit but can be done at any visit) for generation of the induced pluripotent stem cells (iPSCs) from peripheral blood mononuclear cells (PBMC) as described below.

Frequency of blood draw: three time points, Baseline, 1 and 3 month visits.

Blood samples for 35ml collection are for GGT levels: Serum Separator Tube (SST); DNA extraction, Acamprosate blood levels, Metabolomic, Proteomic biomarkers (EDTA tubes), and mRNA (PAXGene tube). The process of spinning and aliquoting the samples will be completed at each site. EDTA tubes that would need to be kept on ice, centrifuged in the cold (NOT frozen) with the plasma aliquoted into 200 ul aliquots which would need to be immediately placed and stored at minus 80 degrees. The current metabolomic platform would include both the amines and neuromodulators. Acamprosate blood drug levels will be plasma aliquoted into 300 ul aliquots and also assayed with these samples. PAXGene tube is placed directly into the minus 80 degree freezer.

An additional 15 ml of blood collected in EDTA tubes for peripheral blood mononuclear cells (PBMC) isolation. The separation and banking of PBMCs will be performed in the laboratory, frozen and stored in liquid nitrogen. PBMC are used to generate induced pluripotent stem cells (iPSCs), a cell type that can self-renew and which can be to differentiated into many cell types from many human tissues.

Review of medical records, images, specimens – Category 5

For review of existing data: provide a date range or an end date for when the data was generated. The end date can be the date this application was submitted to the IRB. Example: *01/01/1999 to 12/31/2015* or all records through *mm/dd/yyyy*.

Date Range: *12/29/2004 to 07/09/2013*
Check all that apply (data includes medical records, images, specimens).

☐ (5a) Only data that exists before the IRB submission date will be collected.

☒ (5b) The study involves data that exist at the time of IRB submission **and** data that will be generated after IRB submission. Include this activity in the Methods section.

☒ (5c) The study will use data that have been collected under another IRB protocol.

☒ Data ☐ Specimens ☐ Data & Specimens IRB #16-004978 "Pharmacogenomics of Treatment Outcomes in Alcohol Use Disorders"

☐ Data ☐ Specimens ☒ Data & Specimens IRB #07-007204 "A Pharmacogenomic Probe Study of Acamprosate: Genes Associated with Response" Mayo Clinic Center for the Individualized Treatment of Alcohol Dependence" (CITA) Recruitment dates: 03/14/2008 to 02/01/2013

☐ Data ☒ Specimens ☐ Data & Specimens IRB #2681-04 "Developing a DNA Repository for Genomic Studies of Addiction" (GOA) Recruitment dates: 12/29/2004 to 07/09/2013

☒ (5d) This study will obtain data generated from other sources. Examples may include receiving data from participating sites or an external collaborator, accessing an external database or registry, etc. Explain the source and how the data will be used in the Methods section.

☐ (6) Video audio recording: *Describe the plan to maintain subject privacy and data confidentiality, transcription, store or destroy, etc.*

HIPAA Identifiers and Protected Health Information (PHI)

Protected health information is medical data that can be linked to the subject directly or through a combination of indirect identifiers.

Recording identifiers (including a code) during the conduct of the study allows you to return to the medical record or data source to delete duplicate subjects, check a missing or questionable entry, add new data points, etc. De-identified data is medical information that has been stripped of all HIPAA identifiers so that it cannot be linked back to the subject. De-identified data is **rarely** used in the conduct of a research study involving a chart review.

Review the list of subject identifiers below and, if applicable, check the box next to each HIPAA identifier being recorded at the time of data collection or abstraction. Identifiers apply to any subject enrolled in the study including Mayo Clinic staff, patients and their relatives and household members.

Internal refers to the subject's identifier that will be recorded at Mayo Clinic by the study staff.
External refers to the subject's identifier that will be shared outside of Mayo Clinic.

Check all that apply:	INTERNAL	EXTERNAL
Name	X	
Mayo Clinic medical record or patient registration number, lab accession, specimen or radiologic image number	X	
Subject ID, subject code or any other person-specific unique identifying number, characteristic or code that can link the subject to their medical data	X	
Dates: All elements of dates [month, day, and year] directly related to an individual, their birth date, date of death, date of diagnosis, etc. **Note:** Recording a year only is not a unique identifier.	X	
Social Security number		
Medical device identifiers and serial numbers		
Biometric identifiers, including finger and voice prints, full face photographic images and any comparable images		
Web Universal Resource Locators (URLs), Internet Protocol (IP) address numbers, email address		
Street address, city, county, precinct, zip code, and their equivalent geocodes		
Phone or fax numbers		
Account, member, certificate or professional license numbers, health beneficiary numbers		
Vehicle identifiers and serial numbers, including license plate numbers		
Check 'None' when none of the identifiers listed above will be recorded, maintained, or shared during the conduct of this study. (exempt category 4)	☐ None	☒ None

Data Analysis

Statistical Analyses and Power

Analysis for Specific Aim 1a

The data that will be used in Aim 1a has undergone quality control (QC) as a part of a prior project (IRB #16-004978). For the analysis of heritability captured by common SNPs, we will first estimate the heritability of different measures of AUD treatment response (e.g. length of sobriety, return to heavy drinking or percent days abstinent) explained by common SNPs using the mixed linear model approach implemented in the GCTA software (104). This approach requires more stringent QC than for standard GWAS. Thus, we will begin by performing additional QC in each dataset, which will include: the exclusion of one of each pair of individuals who share more than 2.5% of their genetic material demonstrating distant relatedness, and exclusion of SNPs with Hardy-Weinberg Equilibrium (HWE) p-value $<10^{-3}$. Because these methods can produce biased results in the presence of population stratification, these analyses will be performed in an ancestrally homogenous subsample, specifically participants of European Ancestry as they represent the vast majority of the full sample (Table 2). Principal Component Analysis (PCA) will be used to identify outliers for exclusion, ensuring an ancestrally homogeneous subset of participants. The analysis of imputed data will also be performed, as described below; for these analyses only SNPs imputed with very high confidence ($R^2>0.9$) will be retained. Following QC, we will use GCTA to calculate the Genetic Relationship Matrix (GRM) and will then use a linear mixed model with residual maximum likelihood (REML) analysis to estimate the variance attributable to the SNPs (i.e. SNP-based heritability) for each treatment

outcome measure. These analyses will include study, and principal components that capture ancestry as covariates. Estimates (and confidence intervals) for the SNP heritability of different outcomes will be compared.

Analysis for Specific Aim 1b
Metabolite concentrations will be log-2 transformed, and associations between baseline metabolite concentrations and the time of abstinence since baseline will be evaluated by linear regression with age and gender as covariates. If Timeline Follow Back (TLFB) data at 3-months is absent, the date of last contact will serve as the day of first lapse unless the subject has reported a lapse at an earlier time point. To study the possible effect of depression severity and liver function, regression models including baseline PHQ-9 scores and LFTs (AST, ALT, and GGT) will be compared with those without to determine whether the metabolites remain significantly correlated with treatment outcome. Participants who provided paired blood samples will be classified as responders (maintenance of complete abstinence) or non-responders (any consumption of alcoholic beverages during the trial period), and their metabolite concentrations at baseline and at 3 months will be compared by two-way ANOVA to detect any significant changes between the two time points and whether these changes differ significantly between groups.

After metabolomic markers associated with length of sobriety (or additional secondary phenotypes) are identified, we will apply a "pharmacometabolomics-informed pharmacogenomics" research strategy. Specifically, we will perform genome-wide association analyses to search for genetic variants associated with metabolites that are associated with acamprosate clinical outcomes. We will then evaluate the association of the identified genetic variants ($p<5*10^{-8}$) with clinical treatment outcomes in all available datasets (including the COMBINE and PREDICT datasets used in Aim 1a). We will also pursue all signals, genes and pathways functionally and mechanistically in order to investigate the biological mechanisms underlying sobriety length and other phenotypes associated with acamprosate treatment response. This is the same approach that we used previously with success to study biological mechanisms contributing to effects of selective serotonin reuptake inhibitors in patients with major depressive disorder (MDD).

Analysis for Specific Aim 2a
Genetic association analyses will evaluate the effect of SNP genotypes on acamprosate and placebo treatment outcomes. Well-established pipelines will be used for genotype quality control and imputation prior to performing the genetic association analyses. We will first perform genome-wide association analyses of the primary outcomes in the acamprosate-treated participants with available 3 month outcomes, using standard approaches. For example, we will evaluate the association of SNPs with 3 month continuous abstinence (yes/no) using logistic regression and with length of abstinence using Cox proportional hazard models. We will first use these models to evaluate the association of the treatment outcomes with covariates, including demographic and baseline clinical characteristics, such as depression and anxiety symptom severity (PHQ-9 and GAD-7 scores, respectively), and liver function tests (LFTs) – aspartate aminotransferase (AST), alanine transaminase (ALT) and Gamma-Glutamyltransferase (GGT). SNP association with the primary treatment outcomes will be evaluated while adjusting for relevant covariates and principal components derived from genome-wide SNP data to adjust for population stratification. As discussed in the Statistics Team Section, secondary analyses will also be performed using the entire sample of participants enrolled into the study ["intent-to-treat" (ITT) analysis]. If the same genetic variants contribute to lack of response in the "completer" group and to early dropout (which is likely the case as early dropout frequently results from relapse), the ITT analysis will have greater power than the "completer" analysis. We will also perform genome-wide association analyses using all participants (acamprosate and placebo), including a treatment covariate, and a SNP-treatment interaction to evaluate whether SNPs are associated with clinical outcomes in a drug-dependent manner (e.g. SNP is associated with acamprosate treatment outcome, but not placebo treatment outcome). We will

also evaluate the possibility of sex-specific genetic effects, or genetic effects that are modified by the presence of comorbidities such as depression and anxiety, by exploratory analyses involving interaction terms with these variables.

Most importantly, we will perform a genome-wide meta-analysis of acamprosate pharmacogenomics using the new data and data from available prior studies (COMBINE, PREDICT, CITA; see Aim 1).

Table 2 Subject Characteristics	COMBINE	PREDICT	CITA
N	1393	426	443
Age, mean(SD)	44.4 (10.2)	45.3 (8.7)	42.1 (11.8)
Sex, N(%) male	955 (69%)	328 (77%)	286 (65%)
Race, N(%) white non-Hispanic	1062 (77%)	426 (100%)	412 (93%)
Baseline Consumption Measures (last 30 days)[1]			
Average drinks per drinking day	13.0 (8.1)	19.4 (11.3)	11.8 (7.7)
% drinking days	72.8 (23.5)	82.1(1.3)	50.7 (29.7)
% heavy drinking days	65.2 (24.9)	79.0 (1.3)	45.8 (29.7)
Number of days since last drink	7.7 (5.3)	22.1 (4.4)	24.8 (16.1)
Medications[2]			
Acamprosate, N	608	172	443
Naltrexone, N	614	169	0
No active drug (placebo or no medication), N	466	85	0
Treatment outcomes[3]			
3 month abstinence from any drinking, N(%)	293 (21.2)	194 (45.5)	132 (29.7)
3 month abstinence from heavy drinking, N(%)	432 (31.2)	210 (49.3)	168 (37.9)

[1]For COMBINE and CITA, baseline consumption is based on 30 days prior to the start of medication, while for PREDICT it is based on 30 days prior to the onset of inpatient treatment, as proposed by Mann and colleagues (84).
[2]For the COMBINE study, the number of participants for each medication may include participants on more than one medication (i.e. groups are not mutually exclusive since the study included acamprosate/naltrexone co-treatment).
[3]Numbers for treatment outcomes are based on the ITT sample (i.e. complete sample) assuming those who dropped out prior to 3 months had returned to heavy drinking.

As with the analysis described above, our meta-analyses will also include analyses of the full set of acamprosate and placebo treated participants, with a treatment covariate and tests of SNP-treatment interaction effects. However, our power estimate for the meta-analysis is based only on the combined sample of acamprosate-treated patients, since the size of this sample will determine the power to detect acamprosate-specific pharmacogenomics effects. Assuming a total sample of 1000 acamprosate-treated participants (ITT), the meta-analysis will provide 80% power to detect associations of SNPs with 3-month abstinence during acamprosate treatment (assume ~1/3 abstain and 2/3 relapse) with odds ratios of 2.07 and 1.80 for SNPs with minor allele frequencies (MAFs) of 0.2 and 0.4, respectively, at a genome-wide significance level of $5*10^{-8}$. We also note that analyses of the full dataset (N≈1500) including participants that received acamprosate or other treatment options will provide greater power to detect genetic predictors of treatment-independent predictors of abstinence in patients with AUD.

We recognize that the available sample has relatively little power to detect the effects of individual SNPs, which tend to be very modest for complex traits such as alcohol-dependence related phenotypes. We will therefore apply more advanced analysis methods to maximize power of the study, including gene-level and pathway-based (gene-set) analyses using approaches such as MAGMA [42], and will evaluate polygenic risk score associations and AUD treatment outcomes.

Analysis for Specific Aim 2b:

Analysis for Aim 2b will use a similar strategy the analysis described above in aim 1b. However, in Aim 2b analyses will be performed on the much larger new sample and will also include assessment of drug interactions to evaluate whether metabolomics predictors of treatment outcome differ between acamprosate-treated and placebo-

treated patients. After identifying metabolites that predict treatment outcomes, we will again apply a pharmacometabolomics-informed pharmacogenomics analysis strategy, but genetic associations will be evaluated in the full sample of patients with genetic and treatment outcomes data, including the existing samples used in Aim 1 and the new samples collected in Aim 2.

Analysis for Specific Aim 3 will be completed in the future utilizing the latest methodology and analyses.

Limitations and Proposed solutions

- The proposed study design may not allow for the separation of the effects of depression and anxiety from the treatment effects of SSRIs, and SNRIs listed on an association between genetic variation and acamprosate or placebo-related treatment outcomes. Separation of those effects would have required an additional placebo arm for antidepressant treatment. We considered this option but concluded it would not be feasible and may negatively affect the attainment of the primary study goal - i.e. identification of genetic markers associated with acamprosate-related treatment outcomes in participants with AUD. Therefore, the difference between the effects related to presence/severity of depression or anxiety and the effects of antidepressant treatment on an association between genetic variation and acamprosate or placebo-related treatment outcomes will need to be investigated in future studies.
- Although the size of the new study sample proposed for the pharmacogenomic analyses in Aim 2 is comparable or exceeds the size of samples collected for the CITA, COMBINE and PREDICTS studies, this sample alone is still unlikely to provide sufficient power to detect significant associations. However, as described above, our goal is to conduct a meta-analysis to identify pharmacogenomic markers associated with response to acamprosate in a combined sample including participants enrolled as part of the proposed center as well as those collected in the COMBINE, PREDICT and CITA studies. This will be the largest pharmacogenomics GWAS in AUD to date. Moreover, the sample will also include a large set of placebo-treated participants (N≈800) allowing for differentiation between acamprosate-specific pharmacogenomic effects from treatment independent predictors of abstinence in patients with AUDs.

We also expect that the application of pharmacometabolomics- and imaging-guided pharmacogenetic analyses of the acamprosate-related outcomes described in Aim 3 will allow for the use of intermediate phenotypes (namely metabolomic and neuro-imaging signatures associated with acamprosate response) and will improve the power for the discovery of genetic variations predictive of response to acamprosate.

Endpoints:

The *primary treatment outcome* will be defined as continuous sobriety (yes/no) during 3 months of treatment.
Secondary outcomes (also assessed during 3 months of treatment) will include:

(1) The number of days until first alcohol use assessed by TLFB.
(2) Number of days until first relapse (≥ 5 drinks/day for men and ≥ 4 drinks/day for women) will be assessed using TLFB.
(3) Cumulative abstinence duration proportion: the proportion of days over the length of follow-up during which participants were completely abstinent from alcohol use, a score range of 0 (drinking continuously) to 100 (maintain complete abstinence) is applied.

MAYO CLINIC

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MAYO CLINIC

Addendum A (Medication Half-life)

Abbreviation Key	
SL: Sublingual	SR: Sustained Release
IM: Mucous Membrane	DR: Delayed Release
IR: Immediate Release	ER: Extended Release
DS: Double Strength	SD: Sustained Delivery
ODT: Orally Disintegrating Tablets	PM: Evening Formulation
LA: Long Acting	IV: Intravenous

Benzodiazepine Generic Drug Name	**Trade Drug Name(s)**	**Half-Life Min/Max**	**Half-Life Breakdown**
Alprazolam	Xanax, Xanax XR, Gabazolamine-0.5, Niravam	10.7-19.7 hours	XR: 10.7-15.8 hrs; Disintegrating: 12.5 hrs; IR: 11.2 hrs; W/alcoholic liver disease: 19.7 hrs; Elderly Population: 16.3 hrs
Chlordiazepoxide (Hydrochloride)	Librium	24-48 hours	N/A
Chlordiazepoxide Hydrochloride/ Clidinium Bromide	Librax	5-30 hours	N/A
Chlordiazepoxide/ Amitriptyline Hydrochloride	Limbitrol, Limbitrol DS	5-93 hours	Systemic: Chlordiazepoxide 5-30 hrs; Amitriptyline: 8-93 hrs
Clobazam	Onfi, Sympazan	36-82 hours	Clobazam: 36-42 hours; N-desmethylclobazam: 71-82 hrs
Clonazepam	KlonoPIN, KlonoPIN Wafers	30-40 hours	N/A
Clorazepate (Dipotassium)	Gen-xene, Tranxene T-Tab, Tranxene-SD, Tranxene	2 days	clorazepate dipotassium: 2.29 hrs; Nordiazepam: 2 days
Diazepam	Diastat, Diazepam Intensol, Valium	Up to 100 hours	Compound: Up to 48 hours (alcoholic liver cirrhosis can prolong 2-5 fold); desmethyldiazepam: Up to 100 hrs
Estazolam	Prosom	10-34.6 hours	10-24 hours; 59-68 year olds: 13.5-34.6 hours (18.4 mean)
Flurazepam (Discontinued in 2019)	N/A	2.3-100 hours	Compound: 2.3 hs; N-1-desalkylflurazepam: 47-100 hrs; N-1-hydroxyethylflurazepam: 16 hrs
Flurazepam Hydrochloride (Discontinued in 2019)	Dalmane	2.3-100 hours	Compound: 2.3 hours; N-1-desalkylflurazepam: 47-100 hours
Halazepam	Paxipam	14-100 hours	14 day use 34.7 hrs; N-desmethyldiazepam: 50-100 hrs (14 day use of halazepam, half-life for the metabolite was 57.9 hours)
Lorazepam	Ativan, Lorazepam Intensol	12-14 hours	N/A

Midazolam, nasal	Nayzilam	2.1-7.2 hours	Compound: 2.1-6.2 hours; Metabolite: 2.7-7.2 hours
Midazolam Hydrochloride	Versed, Seizalam	1.8-6.4 hours	N/A
Nitrazepam	N/A	24-29 hours	N/A
Oxazepam	Serax	5.7-10.9 hours	N/A
Prazepam	N/A	30-200 hours	Mean half-life of 78 hours
Quazepam	Doral	39 hours	N/N
Temazepam	Restoril	3.5-18.4 hours	Mean half-life of 8.8 hours
Triazolam	Halcion	1.5-5.5 hours	N/A

Stimulant Generic Drug Name	Trade Drug Name(s)	Half-Life Min/Max	Half-Life Breakdown
Amphetamine	Dyanavel XR, Adzenys XR ODT, Adzenys XR	11-15.12 hours	D-amphetamine: 11-12.36 hours; L-amphetamine: 14-15.12 hours
Amphetamine Sulfate	Evekeo, Evekeo ODT	7-34 hours	N/A
Benzphetamine Hydrochloride	Didrex	6-12 hours	N/A
Dextroamphetamine	N/A	12 hours	N/A
Dextroamphetamine Sulfate	Dexedrine, Dextrostat, Liquadd, ProCentra, Zenzedi	12 hours	N/A
Dextroamphetamine/ Amphetamine	Adderall, Adderall XR, Mydayis	10-13 hours	N/A
Lisdexamfetamine Dimesylate	Vyvanse	< 1 hr-12 hours	Compound: < 1 hr; Metabolite (dextroamphetamine): Half-life about 12 hrs after giving dose
Methamphetamine Hydrochloride (oral)	Desoxyn	4-5 hours	N/A
Armodafinil	Nuvigil	15 hours	N/A
Dexmethylphenidate Hydrochloride	Focal, Focalin XR	2.2 hrs-3 hrs	XR: 3 hours; IR: 2.2 hours
Diethylpropion Hydrochloride	Tenuate, Tenuate Dospan	4-8 hours	N/A
Mazindol	N/A	30 hrs-5.25 days	Parent Compound: 30-50 hrs; Metabolites: 5.25 days
Methylphenidate Hydrochloride	Concerta, Jornay PM, Jornay ER, Jorany DR, Metadate ER, Methylin, Methylin ER, Ritalin, Ritalin LA, Ritalin-SR	2.5-7 hours	Depends on trade name & release: IR: 2.7-3.5 hrs; Jornay DR/ER: 5.9 hrs, Adhansia ER: 7 hrs; Metadate and Ritalin ER: 2.5-6.8 hrs; Concerta ER: 3.5 hrs
Modafinil	Provigil	15 hours	N/A
Pemoline	Cylert (no longer available)	11-13 hours	N/A
Phendimetrazin Tartrate	Bontril, Bontril PDM, Bontril slow-release, Melfiat, Obezine, Phendiet, Phendiet-105, Prelu-2	2-4 hours	Applies to both IR and SR

Phenmetrazine	N/A	Within 24 hrs	Eliminated in urine within 24 hours after dosing
Phentermine Hydrochloride	Adipex, Atti-plex P, Fastin, Phentercot, Phentride, Pro-Fast, Adipex-P, Lomaira	7-8 hours	N/A
Phentermine Resin	Lonamin	7-8 hours	N/A
Phentermine/Topiramate	Qsymia	20-65 hours	Phentermine: 20 hours; Topiramate: 65 hours
Cocaine	N/A	1 hour	N/A

Opioid Generic Drug Name	Trade Drug Name(s)	Half-Life Min/Max	Half-Life Breakdown
Acetaminophen/Caffeine/ Dihydrocodeine Bitartrate	Panlor-DC, Panlor-SS, Zerlor, Trezix	2-5 hours	Acetaminophen: 2-3 hours; Caffeine: 5-6 hours; Dihydrocodeine Bitartrate: 3.5-5 hours
Acetaminophen/Codeine Phosphate	APAP w/ codeine, Capital w/ codeine, Pyregesic-C, Vopac, Tylenol w/ codeine, Tylenol w/ codeine #3, Tylenol w/ codeine #4	1-4 hours	Acetaminophen: 1-4 hours; Codeine Phosphate: 2.5-3 hours
Acetaminophen/Oxycodone Hydrochloride	Endocet, Percocet, Roxicet, Roxilox, Tylox, Narvox, Magnacet, Perloxx	3.9-6.9 hours	Acetaminophen: ER: 5.8-6.9 hrs, IR: 4.1 hrs; Oxycodone Hydrochloride: ER: 4.5-5.4 hrs, IR: 3.9 hrs
Alfentanil Hydrochloride	Alfenta	90-111 minutes	N/A
Aspirin/Caffeine/ Dihydrocodeine Bitartrate	Synalgos DC	3.5-5 hours	Aspirin: 15 mins; Caffeine: 5-6 hrs; Dihydrocodeine Bitartrate: 3.5-5 hrs
Aspirin/Codeine Phosphate	Empirin w/ codeine	2.9-3 hours	Codeine Phosphate: 2.9 hours; Salicylic acid: 3 hours
Aspirin/Oxycodone Hydrochloride	N/A	5.6 hours	Aspirin: 15 minutes; Salicylate: 2-3 hours; Oxycodone: 5.6 hours
Belladonna/Opium	N/A	3-24 hours	Belladonna: Up to 24 hrs; Opium: 3-10 hrs
Benzohydrocodone/ Acetaminophen	Apadaz	4.33-4.78 hours	Hydrocodone: 4.33 hours; Acetaminophen: 4.78 hours
Buprenorphine	Butrans, Belbuca, Probuphine, Sublocade	11 hours-60 days	Buccal film, subdermal implant, transdermal patch: 24-48 hours; SubQ ER Injections: 43-60 days; Hypoalbuminemia: 11 hours
Buprenorphine Hydrochloride	Buprenex, Subutrex	1.2-35 hours	IV: 1.2-7.2 hours; SL: 31-35 hours

Buprenorphine/Naloxone	Bunavail, Suboxone, Zubsolv, Cassipa	1.9-42 hours	Buccal film: buprenorphine 16.4-27.5 hrs; naloxone 1.9-2.4 hrs; Sublingual film: buprenorphine 24-48 hrs; naloxone 2-12 hrs
Butalbital/Acetaminophen/ Caffeine/Codeine Phosphate	Phrenilin w/ caffeine and codeine, Fioricet w/ codeine	1.25-35 hours	Butalbital: 35 hours; Acetaminophen: 1.25-3 hours; Caffeine: 3 hours; Codeine Phosphate: 2.9 hours
Butalbital/Aspirin/Caffeine/ Codeine Phosphate	Ascomp w/ codeine, Fiorinal w/ codeine	2.9-35 hours	Butalbital: 35 hrs; Aspirin:12 mins; Caffeine: 3 hrs; Codeine Phosphate: 2.9 hrs
Butorphanol	N/A	4-7 hours	N/A
Butorphanol Tartrate	Stadol, Stadol NS	4.56-5.8 hours	N/A
Carisoprodol/Aspirin/Codeine Phosphate	N/A	2-9.6 hours	Compound: carisoprodol 2 hrs; aspirin 15 mins; codeine phosphate 2.9 hrs; Meprobamate: 9.6 hrs; Salicylic acid: 6 hrs
Chlorpheniramine Polistirex/Codeine Polistirex	Tuzistra XR	5-21.45 hours	Chlorpheniramine Polistirex: 21.45 hrs; Codeine Phosphate: 5 hours
Codeine Sulfate	N/A	3 hours	N/A
Codeine Phosphate/ Guaifenesin	Allfen CD, Allfen CDX, Tussiden C, Tusso-C, Virtussin A/C, Dex-Tuss, ExeClear-C, Guaifenesin AC, Guaiatussin AC, Guiatuss AC, Mar-Cof CG Expextorant, Robitussin AC	N/A	N/A
Dezocine	N/A	2.6-2.8 hours	N/A
Difenoxin Hydrochloride/ Atropine Sulfate	Motofen	24-72 hours	N/A
Diphenoxylate Hydrochloride/ Atropine Sulfate	N/A	2.5-4.5 hours	Diphenoxylate Hydrochloride: 2.5 hrs; Atropine Sulfate: 2.5 hours; Diphenoxylic Acid: 4.5 hours
Fentanyl	Duragesic, Subsys, Ionsys	3-27 hours	IV: 3-12 hrs; Sublingual: 5.25-11.99 hrs; Transdermal patch: 20-27 hrs
Fentanyl Citrate	Actiq, Sublimaze, Fentora, Onsolis, Abstral, Lazanda	2.63-24.9 hours	Intranasal spray: 15-24.9 hrs; IM/IV: 219 mins; Oral (troche/lozenges): 7 hrs; Oral (bucal tabs): 2.63-4.43 hrs(100-200 mcg), 11.09-11.7 hrs (400-800 mcg); Oral bucal soluble films: 14-19 hrs; Sublingual tab: 5.02-6.67 hrs (100-200 mcg), 10.1-13.5 hrs (400-800 mcg)
Fentanyl/Droperidol	N/A	2-4 hours	Fentanyl: 2-4 hrs; Droperidol: 2.2 hrs

Hydrocodone Bitartrate	N/A	7-9 hours	Tablets: 7-9 hours; Capsules: 8 hours
Hydrocodone Bitartrate/ Chlorpheniramine Maleate	Vituz	4-24 hours	Hydrocodone: 4 hours; Chlorpheniramine: 21-24 hours
Hydrocodone Bitartrate/ Acetaminophen	Lorcet, Lortab, Vicodin HP, Anexsia, Maxidone, Norco, Zydone, Ceta Plus	3.8 hours	Hydrocodone: 3.8 hours; Acetaminophen: 1.25-3 hours
Hydrocodone Bitartrate/ Guaifenesin	FluTuss XP, ExeCof XP, Extendryl HC, Hydro-Tussin HG, Narcof, ExeClear, Canges-XP, Monte-GHC	4-5 hours	Hydrocodone: 4-5 hrs; Quaifenesin: 1 hour
Hydrocodone Bitartrate/ Homatropine Methylbromide	Hycodan, Hydromet, Tussigon	4 hours	Hydrocodone: 4 hours; Homatropine Methylbromide: Unknown
Hydrocodone Bitartrate/ Ibuprofen	Reprexain, Vicoprofen, Ibudone	4.5 hours	Hydrocodone: 4.5 hrs; Ibuprofen: 2.2 hrs
Hydrocodone Bitartrate/ Pseudoephedrine Hydrochloride	Pancorf HC, Rezira	4-6 hours	Hydrocodone: 4 hrs; Pseudoephedrine Hydrochloride: 4-6 hrs
Hydrocodone Polistirex/ Chlorpheniramine Polistirex	TussiCaps, Tussionex Pennkinetic	4-24 hours	Hydrocodone: 4 hours; Chlorpheniramine: 16-24 hours
Hydrocodone/Chlorpheniramine/ Pseudoephedrine	Notuss-Forte, Hyphed, Hydron PSC, Zutripro	4-24 hours	Hydrocodone: 4 hours; Chlorpheniramine: 21-24 hours; Pseudoephedrine: 4-6 hours
Hydrocodone/Pseudoephedrine/ Guaifenesin	Hycofenix	4-6 hours	Hydrocodone: 4 hours; Pseudoephedrine: 4-6 hours; Guaifenesin: 1 hour
Hydromorphone Hydrochloride	Dilaudid, Dilaudid-5, Dilaudid-HP, Palladone, Exalgo	2.3 hours	Intravenous
Hydromorphone	N/A	11 hours	ER Tablets
Levomethadyl	N/A	35-60 hours	N/A
Levorphanol	N/A	11 hours	N/A
Levorphanol Tartrate	Levo-Dromoran	11-16 hours	N/A
Meperidine Hydrochloride	Demerol, Meperitab	3-48 hours	Compound: 3-8 hours; Metabolite (normeperidine): 20.6-48 hours
Meperidine Hydrochloride/ Promethazine Hydrochloride	Mepergan	N/A	N/A
Methadone Hydrochloride	Dolophine, Methadone HCL Intensol, Methadose, Methadose Diskets, Methadose Dispersible	8-59 hours	N/A
Morphine Sulfate	AVINza, Kadian, MS Contin, Morphabond ER, Oramorph SR, Roxanol-T, Rms	2-15 hours	IV: 2 hours; Kadian XR: 11-13 hours; Kapanol™ SR: 15 hours

Morphine Sulfate Liposome	DepoDur	2-32.9 hours (+/- 24.2 hours)	Epidural: 4.2 hrs (+/- 2.1 hrs)-32.9 hrs (+/- 24.2 hours); Intravenous: 2 hours
Morphine Sulfate/Naltrexone Hydrochloride	Embeda	29 hours	Morphine Sulfate: 29 hours; Naltrexone Hydrochloride: No results
Nalbuphine	N/A	2.2-5 hours	N/A
Nalbuphine Hydrochloride	Nubain	5 hours	N/A
Opium	N/A	48 hours	75% excreted in urine within 48 hrs
Oxycodone	Xtampza ER	5.6 hours	N/A
Oxycodone Hydrochloride	Dazidox, Eth-Oxdose, Oxaydo, OxyCONTIN, OxyCONTIN CR, Oxydose, Oxyfast, Oxy IR	147mins-8.9 hrs	IR: 3.5-4 hrs; Orogastric: 147 mins; Conrolled Release: 4.5-8 hrs; Remoxy R XR: 8.9 hrs (10mg), 6.62 hrs (20 mg)
Oxycodone Hydrochloride/ Naloxone Hydrochloride	Targiniq ER	3.9-17.2 hours	Oxycodone Hydrochloride: 3.9-5.3 hours; Naloxone Hydrochloride: 4.1-17.2 hours
Oxycodone Hydrochloride/ Naltrexone Hydrochloride	Troxyca ER	4-13 hours	Oxycodone Hydrochloride XR: 7.2 hrs; Naltrexone Hydrochloride: 4 hrs; 6-beta-naltrexol: 13 hrs
Oxycodone Hydrochloride/Ibuprofen	Combunox	3.1-3.7 hours	Oxycodone Hydrochloride: 3.1-3.7 hrs; Ibuprofen: 1.8-2.6 hrs
Oxymorphone Hydrochloride	Numorphan, Opana, Opana ER	7.25-9.43 hours	N/A
Promethazine Hydrochloride/ Codeine Phosphate	Phenergan w/ Codeine	N/A	N/A
Promethazine/Phenylephrine/ Codeine Phosphate	Promethacine VC w/ Codeine	2.5-14 hours	Promethazine: 10-14 hours; Phenylephrine: 2.5 hours; Codeine Phosphate: 3 hours
Propoxyphene	N/A	6-36 hours	Compound: 6-12 hrs; Metabolites (norpropoxyphene): 30-36 hrs
Propoxyphene Napsylate/ Acetaminophen	N/A	6-36 hours	Compound: 6-12 hrs; Metabolites (norpropoxyphene): 30-36 hrs; Acetaminophen: 2-4 hours
Remifentanil Hydrochloride	Ultiva	3-10 minutes	Dose related
Sufentanil Citrate	Sufenta, Dsuria	164 min-13.4 hrs	Sublingual: 13.4 hrs; Injection: 164 minutes
Tapentadol Hydrochloride	Nucynta, Nucynta ER	4-5 hours	
Tramadol Hydrochloride	Ultram, Ultram ER, Ryzolt, Rybix ODT, FusePaq, Synapryn, ConZip	5.6-11 hours	Depends on release & trade name: IR: 5.6-6.7 hrs; ER: 6.5-10 hrs; Ryzolt: 6.5 +/- 1.5 hours; Ultram: 7.9 hrs; ConZip: 10 hrs
Tramadol Hydrochloride/Acetaminophen	Ultracet	5-9 hours	Tramadol Hydrochloride: 5-9 hrs; Acetaminophen: 2-3 hrs

Triprolidine/Pseudoephedrine/ Codeine	Triacin C	2.1-10 hours	Triprolidine: 2.1-5 hours; Pseudoephedrine: 4-10 hours; Codeine: no results