SPECIFIC AIMS

Alcohol dependence is one of the leading causes of disability worldwide.[1] As such, the delivery of effective treatment for alcohol dependence could have a profound public health significance. The efficacy of naltrexone for the treatment of alcohol dependence has been established in at least 23 placebo-controlled randomized clinical trials, with only 5 trials failing to show a significant between-groups difference.[2] The demonstration that naltrexone is effective in the treatment of alcohol dependence followed many years of human and animal research investigating the role of the opioid system in mediating response to pain and pleasure. Despite the better group response to naltrexone in clinical trials, there is significant variability in response among individuals treated with medication. While some of this variability is due to differences in adherence and obtaining a functionally significant dose,[3, 4] there remain intra-individual differences in patient response that have not yet been accounted for or predicted. If it were possible to isolate variables that could predict a greater likelihood of positive response to the medication, it would be possible to use the medication with greater certainty and efficiency. This is the basis of much of the contemporary effort in the field of pharmacogenetics.[5] Thus, a critical question remains: for which patients will naltrexone have the greatest benefit?

There is preclinical and human laboratory evidence that a functional, non-synonomous SNP of the mu-opioid receptor (A+118G, Asn40Asp, rs17174829) imparts a significant change in the function of the receptor (see background section). Work in the past few years with our existing database of naltrexone studies has suggested that alcohol dependent patients with one or two copies of the Asp40 variant who were adherent to medication had significantly better treatment response (73.9 %), as measured by the absence of any heavy drinking, than patients with the Asn40 variant (49.0 % response). In patients receiving placebo there was no association between response and genotype, with rates of response similar to those homozygous for the Asn40 allele treated with naltrexone. There are many isolated reports of innovative genetics, and replication has become critical in this field. Importantly, our finding was recently replicated in preliminary analyses of participants in the NIAAA sponsored COMBINE study. As presented at the recent ACNP and RSA meetings and under review for publication at the time of this submission, 87% of those patients assigned to Medical Management with naltrexone who had one or two copies of the Asp40 allele had a positive outcome to treatment compared to a 49% response among those who were homozygous for the Asn40 allele and treated with naltrexone.[6] Such a clinically relevant bio-marker could have an important impact on treatment outcomes as well as the DSM classification system as described recently by Hyman.[7]
Based upon these very promising findings, the aim of this application is to examine prospectively the interaction between a functional polymorphism of the mu-opioid receptor ($A_2118G$ (Asn40Asp)) and response to treatment with naltrexone. A secondary aim of this proposal is to examine the role of the Asp40 allele in alternating the subjective effects from alcohol use in alcohol dependent individuals that have been demonstrated in human laboratory experiments.

To meet these aims, 150 alcohol dependent outpatients with one or two copies of the Asp40 variant of the mu-opioid receptor and 190 subjects homozygous for the Asn40 variant will be recruited and randomly assigned to treatment with either naltrexone (50mg/day) or placebo in a double-blind fashion over a 12-week period. Treatment assignment will be stratified by genotype. Only subjects of European or Asian descent will be recruited for this trial due to the low allele frequency of the Asp40 variant among individuals of African descent (less than 1% allele frequency, www.hapmap.org). Patients will also receive Medical Management (MM), the medical-based intervention used by the NIAAA-funded, multi-site project COMBINE, to support alcohol abstinence while providing pharmacotherapy in a safe, psycho-educational format. The primary outcome measure will be the occurrence of heavy drinking days (as defined by $\geq$5 drinks/day for males; $\geq$4 for females); this measure has become standard in the treatment of alcoholism and provides the most comparability with past and future studies. Recruitment will occur at four clinical sites and will be coordinated at the University of Pennsylvania's Treatment Research Clinic.

Primary hypothesis:

1. Naltrexone - but not placebo - will produce a greater clinical response during the 12 weeks of the trial in subjects with one or two copies of the Asp40 variant (“Asp40 positives”) than in subjects homozygous for the Asn40 allele. Response to naltrexone will be measured by a reduction in the number heavy drinking days (as defined by $\geq$5 drinks/day for males; $\geq$4 for females) during the 12 weeks of the trial.

Secondary hypotheses:

2. There will be an interaction between medication and genotype such that, as compared to the groups on placebo or homozygous for Asn40, Asp40 positive subjects randomized to naltrexone will report:

   a. less “high” from alcohol consumption (on the Biphasic Alcohol Effects Scale), and
   b. the lowest levels of alcohol craving over time (on the Penn Alcohol Craving Scale).

It is acknowledged that most pharmacogenetic interactions have not been this specific or this robust. However, unlike many psychoactive medications, naltrexone is a highly specific
antagonist to only one receptor class, and this polymorphism in the mu-receptor gene is known to cause significant changes in the transcription, translation, and function of that receptor. Equally important to this discussion is the recognition that the clinically important response is not a unique effect of naltrexone on the opiate receptor. Rather, the pivotal variation is in the endogenous opioid (EO) response to alcohol. For those drinkers who have a large EO response, alcohol stimulation is blocked by naltrexone, but alcoholics who lack a strong EO response to alcohol would notice no or limited benefit from naltrexone.

In addition to the scientific aims, the study is designed to provide sufficient information to submit an application to the FDA for approval of an assay of the Asp40 allele to serve as a biomarker and to inform labeling changes for all opioid antagonists approved for the treatment of alcohol addiction. Given this goal, our group, in collaboration with program staff from NIAAA, met with the FDA on February 28, 2007 to ask for a preliminary review of the protocol. We submitted the protocol and a series of questions related to the design and had a dialogue regarding the implications of this proposed study. The FDA group provided feedback in the form of minutes which are included in the appendix. The current application reflects recommended changes from the FDA. Thus the critical elements of design, including the use of placebo and inclusion of an Asn40 allele group, are incorporated as a requirement of FDA approval.

Additional Genetic Analyses: Although we propose a highly specific interaction between treatment with naltrexone and the Asp40 allele, this specificity will be strengthened by demonstrating a lack of effect on other candidate genes involved in the reward pathway. Thus, we plan a formal test of naltrexone treatment response with additional candidate genes involved in the reward pathway, with very specific interest in a class of genes called MORIPs or Mu-Opioid Receptor Interacting Proteins. Finally, we are collaborating with David Goldman, PhD from the NIAAA intramural program; we will provide DNA and clinical assessments from this project and two other open label treatment studies of naltrexone for use in whole genome association studies. The combination of these three trials will yield approximately 600 alcoholic subjects treated with naltrexone and followed prospectively using the same assessments and the same psychosocial treatment platform (Medical Management).

BACKGROUND AND SIGNIFICANCE

In this section, we summarize published findings that have guided the design of the proposed study. We briefly review the current concepts regarding the efficacy and use of naltrexone (NTX) for alcohol dependence. We then present data from preclinical and clinical neuroscience studies supporting a genetic/familial component to response from opioid receptor antagonism. We highlight data demonstrating an association between the Asp40 allele variant and response to treatment with NTX.

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Pharmacotherapy of alcohol dependence

In the past two decades, remarkable scientific progress has been made in the understanding of the causes of alcohol dependence and the development of effective treatments. In December 1994, the FDA approved naltrexone -- the first new medication for alcohol dependence in nearly 50 years-- to be used in conjunction with psychosocial support to treat alcohol dependence. Research has demonstrated that when used in conjunction with a psychosocial treatment program, NTX can improve treatment outcomes, particularly relapse to heavy drinking.[8-15] Other studies have failed to show a significant drug-placebo difference,[16, 17] leading to a conclusion that NTX, while efficacious, is not effective for all patients (see NTX review in appendix). Given that alcohol dependence is a heterogeneous disorder with multiple possible causes, the lack of universal efficacy is not surprising. Indeed, a critical aim for the field is not to find the single treatment that is beneficial for all patients, but rather to determine under what circumstances or for which patients a particular treatment will have the greatest effect.

This application seeks to answer the question: are there particular patients who will benefit most from NTX? Thus, this application is consistent with the NIAAA PA-07-066 entitled “Alcohol Use Disorders: Treatment, Services Research, and Recovery.” One of the specific areas of the PA with particular relevance to this project is “Delineation of patient characteristics predictive of favorable treatment outcome.” Early work in this area has suggested that family history may play a key role in response to NTX in placebo-controlled trials.[15, 18, 19] Based on evidence of a functionally active polymorphism in the mu-opioid receptor, two naltrexone treatment studies have now found post-hoc evidence that the ASP40 polymorphism may have a specific effect on predicting treatment response, while a smaller study did not find such an association. If the findings from these post-hoc studies are replicated prospectively, OPRM1 genotyping may provide a diagnostic test for identifying patients who may be most likely to respond to an opioid antagonist and those for whom other available treatments may be more efficacious. Such a pharmacogenetic approach to treatment has economic implications, as it increases the cost-effectiveness of alcoholism pharmacotherapy via patient-treatment matching.

The role of the mu-opioid receptor in alcoholism

The use of NTX is based on studies in animal models demonstrating a role for endogenous opioids in ethanol reinforcement.[20-22] Animal studies have shown that alcohol is ingested after, rather than during, a stressful event and that there is a rebound reduction in endorphin levels after stress. Moreover, alcohol consumption leads to an increase in beta-endorphin release. Thus for most people alcohol may be most reinforcing during periods when endorphins are particularly low – e.g. after stress. While endorphin release is only one action of alcohol, it is reasonable to think that this may be a part of its reinforcing effects. There may be individuals or animals that are particularly sensitive to the alcohol effects on the endorphin system, and this may be due to an identifiable genetic difference, as suggested by Gianoulakis and colleagues.[23, 24]
Animal studies have also shown that opiate antagonists reduce alcohol preference,[25-28] particularly in strains bred for excessive alcohol drinking[29] and following environmental stressors that elicit excessive alcohol drinking.[30] Clinical studies have demonstrated that NTX may reduce the reinforcing or pleasurable effects of alcohol in social drinkers[31] and in alcohol dependent subjects who slip and sample alcohol.[8, 9] Together, the clinical, pre-clinical and animal data suggest that opioid antagonism may have important pharmacological effects on reducing the reinforcing effects of alcohol and on returns to clinically significant drinking.

Relation between family history of alcoholism and the opioid system.

Supporting a relationship between the function of the opioid neurotransmitter system and family history of alcoholism, Gianoulakis and colleagues demonstrated a greater release of plasma beta-endorphin after an alcohol challenge in non-alcohol dependent adults who are at high-risk (HR) for alcoholism based on family history, compared to low-risk (LR) individuals.[24] Alcohol ingestion led to a dose dependent increase in plasma beta-endorphin only in the HR subjects, despite identical blood alcohol levels. Other investigators have also demonstrated differences in basal levels of ACTH[32] and cortisol in high risk individuals,[33] both of which are partially regulated by inhibitory input from opioid neurons. Decrements in beta-endorphin may reflect a feedback inhibition of release due to a down regulated mu-receptor. Alternatively this may reflect dysregulation of release, as suggested in studies of alcohol preferring mice showing differences in proenkephalin and proopiomelanocortin mRNA compared to non-alcohol preferring mice.[34]

Other studies have also demonstrated differential subjective responses to mu-opioid antagonism by NTX after alcohol consumption. Specifically, King et al. found that NTX blunts the subjective stimulation or “high” experienced after alcohol consumption in HR individuals relative to LR individuals as measured by the Biphase Alcohol Effects Scale.[35] These investigators also demonstrated that a single dose of NTX in HR individuals led to a heightened response of ACTH and cortisol release relative to LR individuals following alcohol challenge.[36] Similarly, McCaul et al. demonstrated in non-alcohol dependent individuals a NTX dose dependent effect on dampening of “liking” and “best effects” compared to placebo after an acute alcohol challenge.[37] They did not find effects of NTX on drunkenness or intoxication, suggesting that the effect was a blunting of the reward/reinforcement of alcohol use. They replicated this work and additionally demonstrated a NTX blunting of ACTH release after an alcohol challenge.[38] These data complement our own center’s experience that demonstrates a blunting of the “high” associated with drinking among alcohol dependent subjects treated with NTX compared to placebo.[39] Using the same methods as this latter study, we are hypothesizing a similar effect related to genotype. In those subjects assigned to NTX, we anticipate that those with the one or two copies of the Asp40 allele will have lower self-reported feelings of stimulation or “high” compared to those homozygous for the Asn40 allele after a lapse of drinking (Hypothesis # 2). Most recently, in alcohol dependent individuals presented a choice of alcohol or other rewards in a “bar lab” experiment, NTX was shown to preferentially
decrease alcohol ingestion in family history positive (FH+) subjects relative to FH- subjects, particularly in males[40].

Role of genetic polymorphisms of the OPRM1 receptor

The role of family history as a predictor of treatment response has lead to speculation that NTX may function differently in genetically predisposed individuals. NTX preferentially binds to the mu-opioid receptor, which is hypothesized to be the principal site of action of the medication. It has been hypothesized that sequence variability in the gene encoding the mu-receptor, OPRM1, may create a receptor with altered expression, structure, or function, and as a consequence increase or decrease an individual’s susceptibility to substance dependence.[41] In particular, there are two polymorphisms found in OPRM1 exon 1 that alter amino acid sequence, A+118G (Asn40Asp) and C+17T (Ala6Val). Case-control studies have been inconsistent in demonstrating an association between OPRM1 sequence variability and the presence of alcohol or drug dependence.[42-56] However, few of these studies are sufficiently large to have > 90% power to detect a small effect (odds ratio of <1.5) in any mode of inheritance with a minor allele frequency of ~ 15%, the allele frequency of the 118G reported earlier by us for individuals of European origin in a study of heroin dependence.[44] The most recent and one of the largest samples did find an association between dependence and the ASP40 allele.[56]

The A+118G polymorphism is of particular interest, since functional effects of the polymorphism have been demonstrated both in vitro and in vivo. Perhaps most compelling is work done on the translation to mRNA, which appears to be altered significantly in the presence of the Asp40 allele.[57] In addition, Bond and colleagues showed that in cell culture, mu-opioid receptors coded by the Asp40 variant bind beta-endorphin and activate G-protein coupled protein potassium ion channels with three times greater potency than receptors coded by the Asn40 variant.[48] Both Wand et al. [58] and Hernandez-Avila et al. [59] found that individuals with one or two copies of the Asp40 allele had greater hypothalamo-pituitary-adrenocortical (HPA) axis activation induced by the opioid receptor antagonism.

Linking the A+118G polymorphism to clinical response

In clinical settings, the Asp40 allele was demonstrated to impart increased sensitivity to an agonist when measured by pupillary response.[60] In addition, several studies now demonstrate an association between the Asp40 allele and the increased requirements for analgesia for pain[61-64] and opioid sensitivity in heroin addicts.[65]
In addiction settings, Smolka et al. [66] showed that individuals with the Asp40 variant displayed greater dopaminergic sensitivity during acute alcohol withdrawal. Moreover, this polymorphism has been linked to clinical response in nicotine dependence in three different studies, which is another disorder involving the dopamine-opioid reward system. [67-69] In accordance with the hypothesis that certain individuals demonstrate a heightened opioid response to alcohol, the Asp40 allele has also been demonstrated to affect both “high” and craving states. Ray and Hutchison demonstrated that the Asp40 polymorphism was associated with greater reports of “high” and “stimulation” during IV administration of alcohol to binge drinkers, further supporting the implications for alcohol dependent individuals.[70] This effect was then blocked by pretreatment with naltrexone.[71] Similarly, Van den Wildenberg and colleagues showed that Asp40 allele carriers had greater cue induced craving when exposed to alcohol.[72, 73] In non-treatment seeking heavy drinkers, McGeary and colleagues demonstrated that subjects with an Asp40 allele taking naltrexone had higher states of craving during cue exposure compared to the homozygous carriers of the Asn40 allele.[74]

Recent clinical studies of naltrexone have demonstrated the importance of genotype as a predictor of treatment response (see review).[75] Work in the past few years with our existing database of naltrexone studies has suggested that alcohol dependent patients with one or two copies of the Asp40 variant had significantly better treatment response, as measured by the absence of any heavy drinking (73.9 % response), than patients with the Asn40 variant (49.0 % response). The response rate among subjects homozygous for the Asn40 allele showed relapse rates on NTX (51.0 %) that were only marginally better than those reported for placebo in combination with psychotherapy.[8, 15] This finding was recently confirmed in analysis of participants in the NIAAA sponsored COMBINE study.[6] As presented at the ACNP and RSA meetings, 87% of those patients assigned to medical management who completed 16 weeks of treatment and who had one or 2 copies of the Asp40 allele had a positive outcome to treatment compared to 49% response among those who were homozygous for the Asn40 allele and treated with naltrexone. These findings may help to explain some of the variability in response to NTX seen both clinically and in randomized clinical trials.

Gelerntner and colleagues found no association between OPRM1 and treatment outcomes among participants in the VA Cooperative study of naltrexone.[76] It is clear from the VA Cooperative study that substantial post-randomization selection bias was present. There was a very significant difference in medication adherence between those participating and those not participating in the genetics subcomponent. As noted by the authors, the sample size was also insufficient. Further, there was a significant naltrexone effect in this self-selected subsample that was not found in the full population. As noted in this article, the discrepancies between the three studies highlight the dangers of relying solely on post-hoc data analysis.
As one or two copies of the Asp40 allele are found in up to 32% of the general population of adults of European descent[44, 48, 50, 52] and in 60% of adults of Asian descent,[42, 77] this polymorphism is also of potential importance on an epidemiological level, since the allele is sufficiently common to be clinically relevant if associated with treatment response. However, the Asp40 variant has a frequency in African Americans of < 5%.[44, 52] Further research focused on the pharmacogenetic response to NTX among African Americans is required.

If the polymorphism causes a change in receptor function, should there be evidence of an association with alcohol dependence? Although there are well-designed case control studies demonstrating weak or no association between the G allele and ethanol dependence, most of these studies have been underpowered to detect the expected small effect sizes with a disease allele frequency of ~ 20%. A treatment endophenotype may be independent of the phenotype of alcohol dependence per se.

What is the role of medication adherence in understanding response to NTX?

In two of the first studies that demonstrated the clinical efficacy of NTX, adherence to the study protocol and to medication was excellent. The high adherence to NTX treatment in these two research studies with alcohol dependent subjects may not generalize to a typical treatment setting, where poor subject adherence can limit treatment effectiveness.[78-80] The role of adherence among patients treated with NTX has been highlighted in several recent publications. Pettinati and colleagues compared the outcomes from two of the prior NTX studies conducted at the University of Pennsylvania/VA Medical Center.[81] For patients who adhered to the prescribed treatment, relapse rates were lower in the NTX group compared to the placebo group (p<0.001). For nonadherent patients, relapse rates were high and comparable in both groups. Similar findings were reported by Chick[11] who showed that because overall treatment adherence was fair (60% of subjects were non-adherent), NTX was not shown to be effective. However, when the results were analyzed for those subjects who were adherent, NTX significantly reduced drinking relative to similarly adherent placebo subjects. These findings suggest that psychosocial programs, which enhance adherence, will be associated with larger treatment effectiveness for NTX, and that adherence to medication has to be incorporated into interpretations of response.

Given the importance of adherence in promoting positive clinical outcomes, we will use procedures, including the psychosocial intervention Medication Management, developed by the NIAAA sponsored COMBINE study, to enhance adherence. Medication Management (MM) uses a number of strategies to increase adherence, including direct feedback on adherence, once daily dosing of medication, and developing a supportive relationship with the health-care provider through frequent visits. All of these aspects of MM have been shown to promote adherence to treatment.[82-87] MM also incorporates aspects of motivational interviewing to
reduce excessive drinking.[88] As noted earlier, we are unable to utilize the depot formulation for this trial due to the cost and lack of support from the manufacturer.

**Summary**

Results emphasizing the value of a pharmacogenetic approach to the use of NTX can have a substantial positive impact on clinical practice. If our pilot results are confirmed, clinicians will have the ability to identify those patients most likely to benefit from a therapeutic trial of NTX for alcohol dependence. Not only will this open the door for identifying patients who would derive specific benefits from NTX, but results may also suggest how to identify patients who will have marginal benefit from NTX. In this way, these patients could be spared exposure and the associated costs of medication and potential adverse effects.

**RESEARCH DESIGN AND METHODS**

**Overall design**

The aims of the study are to test for treatment outcome differences in alcohol dependent subjects randomly assigned to 12 weeks of treatment with NTX (50mg/day) or placebo among those with one or two copies of the Asp40 allele of the mu-opioid receptor compared to those homozygous for the Asn40 allele. Thus, the design of the study is a 2X2 cell double-blind randomization to NTX or placebo stratified by genotype. To meet these aims, 150 alcohol dependent outpatients with one or two copies of the Asp40 variant of the mu-opioid receptor and 190 subjects homozygous for the Asn40 variant will be recruited across the four participating sites.

Patients will be recruited from one of four sites through advertising, established relationships with referral sources, screening in primary care, and referrals from former patients. Patients will be screened and enrolled after meeting minimal inclusion and exclusion criteria designed to maximize the generalizability of the participating study sample. Only subjects of European or Asian descent will be recruited for this trial due to the low allele frequency of the Asp40 variant among adults of African descent. Patients will also receive Medical Management (MM). MM is a medical-based intervention, used by the NIAAA-funded, national multi-site project COMBINE, that supports abstinence while providing pharmacotherapy in a safe, psycho-educational format.[2, 103] Outcomes will be assessed by research staff blinded to treatment assignment and genotype during the course of 12 weeks of treatment. The primary outcome measure will be the absence of heavy drinking (defined by no drinking days of >5 drinks/day for males; >4 for females) as measured by the Time-Line Follow Back method.
Project Timetable

We anticipate being able to randomize 6-7 subjects per month across four sites (approximately 3 Asp40 subjects/month). At this rate, we will be able to finish recruitment in the first 4.3 years of the project. The timeline for study completion is: preparation (months 1-3), enrollment (months 3-52), and data analyses and dissemination (months 36 – 60).

Subject Availability and Recruitment

We are proposing to conduct recruitment at four sites all familiar with our procedures and with conducting clinical trial research. No subjects will be excluded based on gender or economic status. Recruitment of non-institutionalized adults for participation in research is one of the primary strengths of our center. We will develop specific recruitment strategies for each site for assuring an adequate flow of subjects for the study. This would include advertising in local newspapers and radio, placing fliers in clinics that serve appropriate populations, and collaborating with primary care physicians in each system.

Subject Selection

Importantly, we have selected eligibility criteria for the proposed project that will allow us to enroll subjects frequently seen in outpatient alcohol treatment. To this end, most of our inclusion criteria are self-explanatory. In addition, primarily for safety purposes, it is important that the subject has achieved some abstinence prior to starting medication (we will require 2 consecutive days of abstinence). The exclusion criteria are almost all due to safety issues when providing pharmacotherapy. Specific subject eligibility criteria for inclusion are as follows.

Inclusion Criteria:

1) Is male or female and 18 years of age or older; 2) has a current DSM IV diagnosis of alcohol dependence; 3) drank an average of 21 drinks/week in the 60 days prior to treatment and had at least 2 occasions of heavy drinking (5 or more drinks on a given day for men), as measured by the Timeline Followback (TLFB); 4) is of European or Asian descent; 5) has vision, hearing and ability to communicate that are adequate to allow study participation; and 6) successfully completes detoxification as manifested by two consecutive days of no self-reported alcohol use immediately prior to randomization.

Exclusion Criteria:

1) Meets DSM-IV criteria for dependence on any substance other than alcohol or nicotine in the last 6 months; 2) tests positive on the urine drug screen for opioids, cocaine, or amphetamine at
the screening visit; 3) meets current or lifetime DSM-IV criteria for bipolar affective disorder, schizophrenia or any psychotic disorder; 4) has unstable or serious medical illness, including history of stroke, seizure disorder, severe liver disease (AST or ALT > 5X normal at the time of randomization), or unstable cardiac disease; 5) needs treatment with any psychotropic medication (antidepressant, antipsychotic, benzodiazepine, or mood stabilizing medication, with the exception of zolpidem used sparingly if necessary for sleep and oxazepam for alcohol detoxification); 6) is a pre-menopausal female who is pregnant, nursing, or not using a reliable method of contraception; 7) is over age 64 and has evidence of severe cognitive impairment as evidenced by a Mini-mental status exam (MMSE) score<24; or 8) has suicidal or homicidal ideation necessitating inpatient hospitalization.

Gender & Minority Inclusion: Based on prior recruitment success, we plan to recruit 30% females. Although there are no hypotheses specifying gender, the anticipated numbers of females will allow for stratification on these variables in some of the analyses. Given the low allele frequency of the A+118G (Asn40Asp) polymorphism among individuals of African descent, it is not scientifically justified to include these persons in this study. If we were to include subjects of African descent, they would be disproportionately represented in those subjects homozygous for the Asn40 allele and we would be unable to interpret the results from these subjects. We are currently conducting alcohol challenge studies in subjects of African descent to test for genotype by alcohol response relationships using a similar design as Hutchinson and colleagues.[69, 70]

Pre-Screening

Potential subjects will be pre-screened after oral consent is obtained. The prescreening will consist of general questions regarding age, current medications, illicit drug use, and current level of drinking. As clinicians as well as investigators, it is our belief that this is a low-risk procedure with the potential for direct benefit to the patient for which informed oral consent is appropriate. Those who are the appropriate age, are not taking a prohibited medication or illicit drug, and report any recent alcohol use will have a screening evaluation scheduled. Information regarding the number of subjects ineligible will be recorded for tracking purposes.

Written Consent Procedures

Before participation in any assessments, voluntary written informed consent will be obtained in accordance with the local IRB. At the consent session, all assessments, lab procedures, and information on treatment are fully explained (See E. Human Subjects for details). Subjects are asked for permission to digitally record their treatment sessions. Subjects are given information about confidentiality, study payments and under what circumstances they may be prematurely discontinued from treatment. Subjects are told that a blood alcohol content (BAC) reading will be required at each visit and that the reading must be 0.00 to ensure that the subject’s
responses are given in an alcohol-free state -- otherwise the visit will be rescheduled. At the end of the session, subjects take a consent “quiz” that can be re-taken until all of the questions are answered correctly. Subjects are given phone numbers of key project staff and a 24-hour emergency number.

**Screening Assessments (Visit 1)**

Screening is typically accomplished during 2-3 visits over a 7-14 day period, during which time the subject may be receiving medical detoxification. A diagnostician or site investigator will administer the SCID to confirm inclusion diagnoses and the absence of exclusionary diagnoses. The research assistant will administer the TLFB, and other assessments (see schedule of assessments). Urine and blood specimens (including DNA for genotyping) will be collected during this time and the subject will be told if their urine screen is positive for any drug use. If the test is positive, they will be permitted one repeat test, but the urine screen must be negative for them to enroll in the study. Subjects will be asked to authorize research staff to contact one or two “significant others” to help locate subjects if they fail to return for study visits.

Outpatient medical detoxification will be conducted as clinically indicated. Our center has extensive experience in conducting outpatient medical detoxification using a combination of frequent (daily) evaluations with a nurse practitioner and the use of oxazepam as monitored by the CIWA-AR.

**Genotyping**

After the first screening visit, whole blood will be sent to the Penn genetics lab for genotyping. Genomic DNA will be extracted from blood samples by standard methods.[104] The $A_{118}G$ SNPs will be genotyped using the PCR-RFLP method of Gelernter et al.[52] After genotyping is complete, the genetics RA will inform the site if the subject is eligible for the trial. Given that the prevalence of having one or two copies of the Asp40 variant is about 25-30%, we will seek to randomize all subjects of this type, but will only need about 50-60% of the screened subjects who are homozygous for the Asn40 variant. In order to keep the randomization balanced over time, we will randomly select subjects screened who are homozygous for the Asn40 variant. Each year, we will examine the distribution of randomized subjects and adjust the proportion of Asn40 subjects recruited in order to maintain a balanced randomization. All research and clinical staff, including the PI, will be blinded to the genotyping and selection process. Those not eligible for study participation will be referred for additional care.

**Randomization Visit**
After all tests and assessments have been completed for the baseline assessment, the MM therapist will review the Inclusion/Exclusion Criteria to determine whether the subject can begin taking the study medication. If approved, the subject will then be randomized for inclusion in the study and take the first dose.

Randomization Procedures

Subjects are randomly assigned to one of the two treatment conditions based on their genotype (AA, AG, GG) in a double-blind fashion. The first dose of medication is taken at the randomization visit. In assigning patients to one of the NTX conditions, we will use a stratified randomization based on race (Asian/Not-Asian), site, and gender. There has been one unpublished study of a depot formulation suggesting that females have a poorer response to NTX than males. Given the small percentage of females expected in this study (30%), if this were found to be true, it would be important to have gender equally represented across each of the treatment conditions. As gender is unrelated to the ASP40 allele stratification, gender is not likely to be related to the primary hypothesis. Although the number of homozygous Asp40 allele subjects is likely to be low, we will stratify the randomization based on genotyping. This will allow post hoc analyses of main effects but is unlikely to result in a sufficient sample to test interaction effects. Stratification does not weaken the power estimates for the overall sample but allows for post-hoc examinations of gender while maintaining the benefits of randomization. The inclusion of 30% women is consistent with the epidemiology of alcohol dependence as determined in the most recent NIAAA epidemiological study.[105] Our statistician will prepare the randomization sequences to be provided to the genetics RA (see below).

Medication Preparation

Medication is prepared by the research pharmacy under the direction of Ken Rockwell, PhD. NTX and corresponding tablets are purchased by the pharmacy. Subjects will receive 50mg/day of NTX or placebo starting on the day of randomization. The research pharmacy will prepare in advance an equal number of NTX and placebo bottles for the first week of therapy, labeled with a study medication number. This study medication number will be assigned so that it will not correspond to the blind. These “starter” bottles will then be stored at each site and available on the day of randomization. In this way, the research staff at each site will be blinded to the contents of any individual packet and the medication number on the packet cannot be deciphered. This is a similar method used in most industry trials. Local pharmacy involvement will be minimal.

At the completion of the baseline assessment, the MM therapist will provide the patient with a MEMS cap with instructions on how to use it on the pill bottle (see below for more information on MEMS caps). The patient will be informed that the MEMS cap counts the number of times the pill bottle is opened and that it will be collected at each follow-up assessment visit. We will
use information from the MEMS cap to provide feedback to the patients (either negative or positive) as a way of enhancing their treatment adherence.

**Protection of the Blind and Medical Emergencies**

All research staff involved in the study will be blind to the status of subjects with respect to genotype and to their receiving medication or placebo treatment. Given that staff will have knowledge about the study and the potential benefits of NTX based on genotype, it is important that the genotype of any individual remain blinded to the patient and staff involved in the care of that patient. On the day of randomization, the MM therapist will call a central number in the genetics lab requesting a medication number for a specific subject. The RA in the genetics lab will provide the nurse with a medication number that corresponds to the appropriate bottle of medication based on the above randomization procedure (the medication numbers will be assigned non-sequentially). The genetics RA will keep a log of patient assignments and notify the pharmacy of each randomization. Both the genetics RA and the research pharmacy will keep the codes linking subjects’ ID numbers to randomization in a locked drawer.

In most cases, an adverse event will not necessitate the breaking of the blind. If an emergency necessitates that the blind be broken, the pharmacist will break the blind only on the authority of the Principal Investigator. Investigators can reach the pharmacist 24 hours a day by beeper and can access the code almost immediately. The research design includes placebo-controlled, double-blind random assignment of subjects. Experience with other studies conducted at the TRC with NTX indicates that there is a low incidence of side effects, which allows the double-blind to be effectively preserved.

**Psychosocial Interventions**

All subjects will receive regularly scheduled manualized adherence enhancement therapy for alcoholism. Sessions last approximately 25-30 minutes, and are focused on patient adherence and current functioning.

During the initial MM visit (done on the day of randomization, see Table 2), the MM therapist provides feedback to the subject about their biopsychosocial status based on selected screening and baseline information collected as part of intake. This will result in more clinician-patient discussions about the nature of alcohol dependence and its relevance to the subject. The MM therapist then educates the subject about NTX, the dosing regimen, how to watch for and manage potential side effects and how to prevent medication nonadherence. Subjects are then given their first dose of study medication (50 mg/day of NTX or placebo). At visit 2 and subsequent visits, the NP will inquire about tolerance to the medication. If side effects occur, the
MM therapist may reduce the dose. If the side effects subside, subjects can be re-challenged or kept at a lower dosage. Study medication is provided to subjects in well-marked pill bottles that contain one month of tablets. Subjects are asked to return these bottles at their next MM visit, empty or not, and they will receive $5.00 each week that they bring back the bottle for adherence checks.

After the initial MM visit, subjects will meet weekly (for the first 8 weeks) and then biweekly for 15-30 minutes for a total of 11 visits, primarily to dispense study medication and assess safety (adverse events), treatment adherence (pill counts and visit attendance), and drinking status. The content of each visit is fully outlined in the MM manual (see appendix) and is briefly outlined in the following tables.

Table 2. Medical Management (MM): Content of Initial Visit
- Review with patient their biopsychosocial status using the Clinician Report from the MM manual
- Discuss with patient the role of the MM clinician: Safety, Education, Support
- Educate patient about his/her alcohol disorder and about the medication, i.e., NTX
- Record baseline safety information and give first dose of NTX
- Discuss importance of pill adherence, develop a plan for medication adherence
- Encourage attendance at MM visits and community self-help support groups

Table 3. Medical Management (MM): Content of Follow-up Visits
- Brief check on medical functioning
- Determine patient status re: drinking, pill taking
- Give support and advice based on patient status

MM Therapist Recruitment and Training

MM Therapists, typically nurses, will be responsible for interacting clinically with the patients and their families. These individuals have been selected in part for their experience with and sensitivity to the kinds of patients and practices to be used in the study. Both of the NPs at the TRC site that we have named on this application are actually MM certified (for COMBINE from New Mexico site; Dr. Miller). During the study, they will receive biweekly supervision of all cases by Dr. Dundon. Training and supervision for the other sites will be based on our experience at the TRC. The training will focus on the clinical skills and pharmacological knowledge needed to manage individual cases within the parameters of MM. The training will also aim to impart a high level of personal responsibility for the welfare of study subjects. We have modeled our training and quality control procedures from our experience in the NIAAA

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COMBINE trial. Key components to delivery of the treatments include standardization (we are using manualized treatments), selection of appropriate staff, certification, monitoring, and documented fidelity.

Dr. Dundon will meet with the MM staff every other week (outside sites will join by conference call) to address problems or review parts of digital recordings. Just prior to initiation of the project and on an annual basis the MM therapists, project coordinator, and PI from each site will meet in Philadelphia for training and project management. At the beginning of the study, Dr. Dundon will be required to listen to sessions one and three of two patients per MM therapist. During the trial, Dr. Dundon will be required to listen to two recordings per week for each NP. The recordings will be rated for therapeutic alliance factors and clinician adherence to the treatment. A record of how often recordings are reviewed by supervisor will be kept, in addition to a record of attendance at supervision meetings. These records will help to ensure capable content and attention to overseeing each psychosocial treatment. Monitoring of treatment is conducted using digital recordings of all psychosocial treatment sessions and rated by Dr. Dundon. If therapeutic drift is recognized, the MM therapist will be informed during supervision and will have additional recordings monitored over the next 3 subjects. If the MM therapist continues to waiver from the MM manual we will consider withdrawing this MM therapist from the trial. Ratings will follow the format developed for the COMBINE study. These ratings will be used to describe therapeutic drift over the course of the study and between MM therapists.

Study-specific policy for managing clinical deterioration and Adverse Events

Our experience suggests that some patients will relapse and require medical detoxification during the course of the study. The MM therapists and the PI have extensive experience providing outpatient medical detoxification. In the event of significant relapse, we will allow two additional medical detoxifications. If the patient requires a third medical detoxification, we will strongly recommend that the patient to seek additional treatment at an inpatient or an Intensive Outpatient Program (IOP). These patients can continue in their treatment in the study. We will record "other" treatment with the Treatment Services Review (TSR) and, if relevant, with Concomitant Medication Report Form.

Side effects can be treated by lowering the dose of study medication or discontinuing it, if necessary. Study medication will be discontinued in subjects who cannot tolerate a minimum dose of study medication, become pregnant, or choose to discontinue study medication or the study for any reason. All subjects, when discontinued from study medication, will be given a final safety evaluation and asked to return their study medication. At any time, subjects who are acutely suicidal will be immediately referred to appropriate psychiatric treatment. Subjects not suicidal but whose depressive or other psychiatric symptoms do not remit by any of the study methods available, e.g., restarting NTX or increasing the frequency of MM, will be referred for
appropriate clinical treatment, and can continue in the trial. We will record "other" treatment with the Treatment Services Review (TSR), and, if relevant, with the Concomitant Medication Report Form.

Study Discontinuation (early)

All subjects who will be randomized are included in the data analysis of this treatment trial, regardless of whether the subject discontinues treatment. That is, they will be included in the analysis of the trial data (intent-to-treat) and will continue to be contacted for research visits. Subjects, therefore, will not be dropped from the study, unless they specifically request that they not be contacted, or the subject cannot be located for assessment by the 12-week follow-up. Patients randomized who are discontinued from medication will continue to be followed in the study until the completion of the 12 weeks and thus we will continue to monitor the adverse effects that caused the discontinuation. The reason the subject is discontinued from the clinical trial or from study medication and any referrals that are made are documented on the Final Evaluation Form in the subject casebooks.

Reasons for Discontinuing Subjects from Medication Treatment

Given the nature of the study, some patients may need to be discontinued from study medication. All reports of adverse reactions to the study medication will be assessed by Dr. Oslin with regard to intensity. Subjects will be discontinued from the study medication under any of the following circumstances:

- Serious or persistent complaints of adverse effects that are likely due to the study medication.
- Any condition under which the study psychiatrist finds NTX to be a hazard to the subject (i.e. pain requiring the use of narcotic medication or evidence of hepatic insufficiency)
- Development of an intercurrent medical illness or condition that requires hospitalization or would be jeopardized by the continued administration of NTX.
- Emergence of another substance abuse problem which necessitates inpatient admission or a more aggressive treatment than provided by the protocol.

Study medication can be restarted after consultation with the research physician for patients who 1) return to treatment after a period of absence, or 2) return to treatment after inpatient hospitalization. Restarting medication is contingent upon clinical need and continuing to meet inclusion and exclusion criteria.

Study Treatment Completion (end of 12-week trial)
At the successful conclusion of the trial, patients will be given an option to continue on NTX in an open label fashion. We will provide a 1-month prescription for patients and assist in finding appropriate aftercare. For those who do not wish to continue, the medication will be stopped at the end of the study. There are no known risks for stopping NTX at the dosages used in this study and thus there is no known advantage of tapering the medication. After discontinuing medication for any reason, patients are encouraged to contact study staff if there are persistent adverse effects. Treatment will not be offered after the end of the treatment phase of the trial. However, efforts will be made to establish follow-up care.

Outcome Assessments

Given the importance of adherence in the interpretation of our findings, we are particularly mindful of the burden of intensive and frequent research assessments. The battery of tests used for this study is brief and focuses specifically on the aims of the project. The research assistant will assess subjects on the same schedule as the MM therapist (weekly for 8 weeks, then biweekly). These scales also match those completed in the ExTENd trial and the ACM study, allowing for use in the WGA analysis.

The outline of assessments is divided into two parts, with those instruments directly relating to outcomes being administered by a trained Research Assistant (RA). These assessments will be used in all outcome analyses. The RA will administer the instruments included in Table 4 according to the outlined schedule. We estimate that each assessment will last no more than 60 to 90 minutes. In addition, the MM therapists will collect clinical data on the content and process of the therapeutic interaction. The data collected by the clinical staff will be used as a validity check of the RA-obtained data and information on safety.

Table 4. Research assessments and measures

<table>
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<tr>
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### Description of Specific Instruments

The instruments to be used in this study have been used by the CSA in several clinical trials. Moreover, many of these instruments are standard across intervention trials. Therefore, we will only describe in brief detail the assessment instruments and their relationship to the aims of the study. The assessment battery will include instruments that will identify functional impairment, physical illness, psychiatric diagnosis, level of current and lifetime alcohol use and severity of alcoholism.

#### A. Diagnosis

i. **Structured Clinical Interview for DSM-IV (SCID).**[106] The SCID-IV is a 45-minute, semi-structured interview that yields current and lifetime DSM-IV Axis I diagnoses for the major psychiatric disorders, including alcohol dependence.

   ii. **Family Informant Schedule and Criteria (FISC).**[107] is an extension of the Family History and Research diagnostic 25-minute structured interview designed to assess family history of psychopathology and psychoactive substance abuse or dependence in first- and second-degree relatives. We have elected not to complete diagnostic interviews of relatives as would be expected in a genetics study. Rather, we are basing family history on subject report as done in prior trials of high-risk individuals and in NTX studies.[15, 18, 35, 36] In this manner, we can replicate these prior findings and link this methodology to our genotyping.

#### B. Alcohol Use

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i. **Time-Line Follow-Back (TLFB).** The TLFB will be used at baseline and each follow-up to assess drinking behavior and time spent in controlled environments, such as treatment settings and prison.[108] The TLFB done at baseline will cover the 90-day period prior to study entry.

ii. **SIP (Short Index of Problems).** The SIP is a 15-item questionnaire designed to measure adverse consequences of alcohol abuse in five areas: interpersonal, physical, social, impulsive, and intrapersonal. The SIP is a brief version of the DrInC recommended in clinical trials as a measure of consequences of drinking.[109] This measure will be used as a secondary outcome measure and potential baseline covariate.

iii. **Alcohol Dependence Scale (ADS).** The ADS[110] is a 5-minute, 25-item self-report of alcohol use (?) severity in the year prior to treatment. The ADS has good reliability and validity.[111] This measure will be used as a secondary outcome measure and potential baseline covariate.

iv. **Subjective High Assessment Scale.** This questionnaire assesses the level of euphoria experienced after ingestion of alcohol in contrast to sedation and intoxication and has been shown to associate with certain genetic traits. This will be used in the WGA analysis.

vii. **Penn Alcohol Craving Scale (PACS).** The PACS measures the degree of internal craving or thoughts regarding alcohol use.

v. **Obsessive-Compulsive Drinking Scale to Measure Alcohol Craving (OCDS).[112-114]** The OCDS is a 10-minute, 14-item subject report of the past 7 days of obsessive and compulsive alcohol-related behaviors in the severity, frequency, and intensity of urges to drink. The OCDS will be used in assessing the role of craving as a secondary outcome.

vi. **Treatment Opinion.** This 5-item instrument queries both the research NP and patient as to their opinion regarding the blinded medication.

vii. **Biphasic alcohol effects scale (BAES).[115]** The BAES is a 14 item validated measure of the sedative and stimulating effects of alcohol consumption. This scale was previously used in demonstrating the effects of NTX on blunting the stimulating effects of alcohol.[35] The scale will be given at the day of randomization (with a minimum of three days abstinence) and at each research visit. The follow-up ratings ask the participant to retrospectively rate their feelings during the most recent drinking period. We also considered using daily diaries or Interactive Voice Recordings to collect this information in greater proximity in time to the consumption of alcohol. However, we felt that this method was unreliable in that it required subjects to recall or record feelings during a window of time immediately following alcohol use.

C. **Comorbidity**
i. **Fagerstrom Scale.** The Fagerstrom Scale\cite{116, 117} is a 5-minute, 8-item self-report instrument designed to measure physical dependence on nicotine. The validity and reliability of this scale have been supported in numerous studies.\cite{116, 118, 119} Scores range from 0 (no dependence) to 11 (high dependence).

ii. **Mini-Mental Status Exam (MMSE).** The MMSE has been widely used in both clinical assessment and research. The instrument is designed as a cognitive screening instrument for exclusion criteria.\cite{120}

### D. Functional Assessment

i. **Medical Outcomes Study Short form (SF- 12).** This scale is used in health services research as a measure of quality of life outcome. The scale is divided into subscales such as physical functioning, role functioning, and social functioning. The scale will be a primary outcome measure of functional capacity.

### E. Treatment Services Utilization

i. **Treatment Services Review (TSR).** The Treatment Services Review (TSR)\cite{121} will also be used to collect data on in-program and out-of-program treatment services received since the last assessment.

### F. Adverse events

i. **Systematic Assessment for Treatment Emergent Effects-General (SAFTEE-GI).** The SAFTEE-GI\cite{128} is a 10-15 minute, semi-structured interview designed to assess and track the onset, course, and severity of adverse effects. A modified version of the SAFTEE is conducted at each visit in order to monitor treatment related adverse events. In addition, vital signs and weight will be routinely assessed.

### G. Laboratory Analysis

i. **Carbohydrate Deficient Transferrin (CDT).** CDT is reported to have approximately 80% sensitivity and specificity for detecting alcohol consumption greater than 50-60 g/d.\cite{129, 130} Dr. Raymond Anton will conduct CDT assays at the Medical University of South Carolina. Unpublished data from our center demonstrates that the relationship between CDT and alcohol use is unaffected by Hepatitis C infection, while AST, ALT, and GGT are all affected. Given that 15-20% of the alcohol dependent patients seen in our center have Hepatitis C, CDT may be a more reliable biological marker of drinking.
ii. Liver Function Tests (LFTs). LFTs are measured to assess hepatocellular injury that may be associated with the acute or chronic abuse of alcohol and other drugs. We are collecting AST, ALT and bilirubin for safety purposes when subjects enter the trial and for following subjects while taking medication. We are collecting GGT primarily to monitor outcome.

iii. Chemzymes, urine pregnancy tests and CBC. Chemistry labs are drawn to monitor for adverse events and for study inclusion criteria. Female subjects will receive a pregnancy test at study entry.

iv. Urine Toxicology and Breathalyzer. EMIT will be used to detect the following drugs: opiates, cocaine, benzodiazepines, and marijuana. Breathalyzer readings will be recorded at each study visit.

v. Genotyping (see above section on genotyping).

vi. Beta-naltrexol level. Published research has indicated that serum levels of beta-naltrexol (a metabolite of NTX) are correlated with response among alcohol dependent subjects[4] and with subjective response in heavy drinkers.[37] Moreover, beta-naltrexol levels are marginally correlated with the dose of NTX. Therefore, beta-naltrexol levels will be used to examine the variability of response to fixed doses of medication and more specifically to confirm adherence to medication. We will collect serum at 4, 8 and 12 weeks of treatment.

H. Clinician Administered Assessments

The following assessments are collected by the clinical staff as measures of clinical outcome, safety and as comparisons to the research assessments.

i. Clinical Global Impression and Severity. The NP will record a Likert scale clinical global impression of treatment improvement and severity of illness at the end of each visit. We will use this in an exploratory manner to evaluate each clinician’s assessment of outcome.

ii. Concomitant Medications. The NP will monitor and record the use of all concomitant medications. These are collected to monitor for safety.

Compensation

In our experience, compensation for time and effort required by research procedures enhances completion of study data and thus directly affects the ability to interpret research results. All subjects will receive $10 for completing the screening assessments, $15 for each of the research visits during the trial (including the randomization visit with $5 contingent on bringing in their pill bottle with the MEMs cap) and $40 for the end of trial visit. Thus, the maximum reimbursement a patient can receive is $230.

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Potential Problems

Will nicotine affect alcohol use?

Since 70% of alcoholics are heavy smokers, it is not feasible to eliminate them from alcohol studies. Thus, we will record if subjects smoke, whether they are dependent, the severity of their dependence (via the Fagerstrom scale), and how many cigarettes are smoked in the 90 days pre-treatment and during the trial. We will utilize these variables in the data analyses when we are looking at treatment outcomes for reducing depressive symptoms and amount of drinking. As noted previously, there is some evidence that success of smoking cessation is moderated by the presence of the Asp40 allele. As we are stratifying by the allele, this will distribute the effect of smoking cessation across the groups.

Defining response and adequate exposure.

We have chosen to establish response after 12-weeks of treatment and to compare that response in a 2X2 design. We acknowledge that response during this brief treatment period might overestimate “placebo” response. However, if this effect occurs it is in the direction of a more conservative estimate of the interaction between medication response and genotype. We have defined an adequate exposure to the medication as taking >80% of the medication over the entire 12-week period. We acknowledge the arbitrary nature of this definition and plan to explore other potential thresholds post-hoc. We chose this threshold based on our experience with our pilot study and on the recognition that adherence in our previous trials has been bimodally distributed. As noted in the pharmacogenetics paper, when selecting adherent subjects, pills were taken for 93% of the study period. As previously stated, our aim was also to define a threshold of effect rather than a measure of high adherence. Moreover, depending on the pattern of non-adherence and based on PET data and blockade of morphine effects showing prolonged central nervous system activity[131, 132], some level of non-adherence should be tolerated without a decrement in efficacy.

Therapist effects

There is a relatively consistent literature documenting the importance of the alliance between therapist and patient.[133, 134] This has led to the practice of having a minimum of 2-3 therapists per condition in intervention trials. We do plan to have several MM therapists involved in the trial and would anticipate over the course of the study the addition of one or more MM therapists through the process of attrition. Despite this, there will inevitably be some nesting of therapists within site that cannot be controlled. We will track the dyad of patient and therapist and will be able to examine effects post-hoc. We will also have information about each therapist including experience, demographics, recovery status, and degree of adherence to the algorithm.[135]
Site effects

The planned analysis will include site as a covariate. However, based on our ongoing experience with ExTENd and the results from the COMBINE study, site is not anticipated to produce a three way interaction (i.e. we do not anticipate that the naltrexone by polymorphism interaction will vary across the four sites). Our experience between the VA and University of Pennsylvania does not suggest differences in baseline severity or in demographics between these two sites, except for the expected differences in gender. For instance, the percent of days of heavy drinking among veterans in the 90 days before randomization is 68 ± 28 compared to 71 ± 29 in subjects recruited from the TRC. Intervention differences due to site could occur if there were site-related differences in therapists’ effects, rates of adherence, or psychosocial treatment. However, randomization and maintenance of the blind, together with the population similarities, should ensure that adherence rates are similar across the sites. In addition, all therapists from all sites will be trained at the Philadelphia site. Finally, the psychosocial treatments administered at each site will be designed, monitored, and supervised from the Philadelphia site. In summary, we expect that these measures will reduce the site to site variations to negligible levels. As a final safeguard, we have planned for a 10% increase in the sample size, to allow for small site to site variation.

Homozygous vs. heterozygous states

We will stratify the randomization based on whether subjects are homozygous or heterozygous for the Asp40 allele. However, the primary hypothesis will combine these groups. This was discussed at some length at the FDA meeting, and although the most rigorous design would oversample homozygotes, this is not practical given the allele frequency. We will be able to examine main effects of medication across the stratified sample. Moreover, the results from the prior trials and the effect size estimates are driven mostly by subjects heterozygous for the Asp40 allele. Finally, there is only weak clinical evidence from our one study of adults of Asian descent suggesting a clinical difference between having one or two copies of the Asp40 allele.[42] The paper resulting from that study found differences at baseline but did not address treatment differences. Given the lack data with which to conduct sample size estimates, a proposal hypothesizing a distinction between homozygotes from heterozygotes would have to be considered preliminary.

Data Analysis

The data analyses will be performed by CSA data analysts, supervised by the PI and by Dr. Lynch. Dr. Lynch will personally perform the more complicated analyses. Prior to performing analyses, standard data screening/cleaning procedures will be applied.[136] These procedures will include: (1) screening the data for data-entry errors, (2) checking for outliers, (3) assessing the extent and pattern of missing data, and (4) checking that appropriate assumptions of normality are met whenever necessary. Some baseline and demographic variables will be
included as explanatory variables in analyses, as they may help reduce error variance. Because of the size of the sample, it is unlikely that the randomization will result in significant imbalance of the distributions of demographic or other variables across the treatment groups. However, the randomization will be checked by comparing the groups on relevant background variables. The comparisons will use analyses of variance for continuous variables and log-linear models for discrete or ordinal responses. Variables on which the groups show significant differences may be included as covariates in later analyses. These analyses will be supplemental to the main analyses, and will serve to assess the sensitivity of the main results to possible randomization imbalances. In all analyses, the assumptions underlying the application of all the statistical methods that are used will be examined, principally through the use of standardized residuals, influence diagnostics, and graphic displays.

We will use selection models[137, 138] to examine the effects of missing data. We will explicitly model the probability of dropout at a time point as a function of baseline characteristics and responses at previous time points, using a logistic regression model, and incorporate the predicted probabilities into the main analysis. Any attempt to model the drop out process requires us to make modeling assumptions about it, none of which can be tested on the observed data. While this was originally seen as a controversial aspect of the approach[139], it has become standard practice to perform the analyses under a range of different assumptions, and to assess the sensitivity of the results to them.[137] For example, to assess sensitivity to the normality and missing data assumptions of the random effects models, we propose to compare results based on the sandwich and information-based standard errors under the random effects models, alter the random effects distributions using Proc Nlmixed, and to implement the GEE methodology in estimating population-average models without random effects, using Proc Genmod in SAS. The GEE procedure is more robust to mis-specification of correlation structures than the mixed effects approach, but not with respect to missing data assumptions (missing at random versus missing at random, respectively).

The primary relative efficacy comparisons in the trial will be based on the intent-to-treat principle.[140] To that end, at the time of enrollment the importance of follow-up assessments will be stressed to all subjects independent of the importance of treatment adherence. Every attempt will be made to obtain outcome data for all patients. Using these methods, we have been able to obtain outcome data on 85% of randomized subjects during a 12-week trial. Although the investigators have developed rigidly defined follow-up techniques, have trained RAs dedicated to conducting follow-up assessments, and will take extreme caution in scrutinizing the data to avoid missing data on forms, inevitably there will be some missing data. Excluding nonadherent subjects and subjects lost to follow-up in “completer” analyses can lead to biased assessment of both treatment efficacy and treatment effectiveness.[141, 142] Given the importance of adherence to medication in defining an interaction between medication assignment and genotype, adherence will be measured using MEMs caps and monitoring of beta-naltrexol levels. These sources will be used to define variables representing
adherence/non-adherence over the 12 weeks of the treatment period. For the primary outcomes, we will perform extensions of instrumental variable regression analyses presented by Nagelkerke et al.[143] Here the instrumental variable is randomization, which under certain assumptions will control for unmeasured bias when estimating the effect of NTX relative to placebo in those who adhere to their assigned medication. In our prior clinical trial of NTX (NAL-4), preliminary use of this methodology has shown promise in better understanding the role of adherence in the primary outcomes. Because the methods are still relatively new, we will supplement them with less formal considerations of the role of adherence. An adequate exposure (adherence) to medication will be defined as taking medication on more than 80% of the treatment days. We will assess the influence of adherence by including the exposure variable as a main effect and as an interaction effect in the main analyses. These analyses can be regarded as a simple pattern-mixture approach[144] and will serve as a sensitivity analysis for the instrumental variable analyses described above.

**Hypothesis 1:** The paramount aim of this study is to examine the relative efficacy of NTX versus placebo in preventing relapse to clinically significant drinking in subjects with one or two copies of the Asp40 allele versus those homozygous for the Asn40 allele. The primary outcomes for Hypothesis 1 will be binary indicators of relapse to heavy drinking (defined by no drinking days of ≥5 drinks/day for males; ≥4 for females, as measured by the Time-Line Follow Back method) over each of the 12 weeks of the trial. A generalized estimating equations (GEE) model[145] will be used to compare the log-odds of relapse between the four groups. The “robustness” of the GEE approach to mis-specification of the within-subject correlation structure comes at the cost of requiring larger sample sizes for stable estimation. Based on the work of Lipsitz et al.[146] the sample size in this study will be more than adequate for the method to be valid. The explanatory variables will be 0-1 variables indicating polymorphism group, NTX group, and their interaction, together with contrast variables representing time effects, as well as the factor representing the site. Our primary interest is in the interaction of NTX group and polymorphism group, but we will also examine group by time interactions to test for differential trends over the three months, prior to examining lower-order effects. We will also check for a site by naltrexone by polymorphism interaction, although our power for such an effect is low.

As required by the FDA, we will examine for discrepancies between self report data and increases in either GGT or CDT levels or the presence of a positive breathalyzer. We will consider a change in CDT or GGT levels of greater than 10% of baseline as representing an increase. If there is a discrepancy, the subject will be considered to have relapse for that time interval. It is noted that this methodology is relatively novel and therefore we will work with the FDA on defining these parameters to meet their objectives. As noted above the primary analysis considers each time period as a binary outcome so that these objective biomarkers can be modeled without difficulty.

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Secondary hypotheses.

**Hypothesis 2:** Weekly administration of the BAES will ensure that subjective assessments of stimulation due to drinking will be obtained reasonably soon after the drinking episode (Hypothesis 2a). When drinking has actually taken place since the last assessment, the response on the BAES “high” scale will be continuously distributed; if no drinking has taken place, the response will be zero. In the absence of such zero values, the hypothesis could be tested by comparing the groups using a mixed effects model for “high” score across the 12 weeks of the study. To incorporate the zero values into the analyses, we will use models and software developed by Olsen and Schafer.[147] These models have two components: a logistic regression model for zero versus non-zero values, and a linear model for distribution of non-zero values, with random effects in both models. The explanatory variables for each model will include NTX group, polymorphism group, and their interaction, together with the time and site terms as described above. The regression coefficients of the linear model will directly address Hypothesis 2a. For Hypothesis 2b, the PACS will be measured on the same schedule as the BAES, but will not exhibit the same degree of zero inflation as the BAES, since any response is possible whether drinking has taken place or not. We will test hypothesis 2b using a linear mixed effects model, with the main explanatory variables being naltrexone group, polymorphism level, and their interaction, site, and terms to represent time trends across the repeated measures. As it is possible that the PACS scores will be skewed towards lower values, we will examine whether log- or square-root-transformations are required before interpreting these models.

Exploratory analyses:

The analyses described above will address the principal research questions of the study. As part of our consideration of adherence questions, and because it is of independent interest, we will compare the groups on adherence to medication and on attendance to therapy visits. We will also examine the relative incidence of a specific set of adverse events, such as nausea and headaches. We will also compare the groups on change in quality of life as measured by the MOS SF-36. This yields continuous scales, and the comparisons will be performed using mixed effects models. In light of the descriptive nature of these analyses, we will compare the various scales separately, rather than as part of an overall “factor.” We will also consider alternative drinking measures: craving; abstinence from any drinking, which will be analyzed in much the same way as described for abstinence from heavy drinking in Hypothesis 1; and time to first day of heavy drinking, which will be analyzed using Cox regression models for censored responses. We will use the FISC to determine the subjects with paternal history of alcohol problems together with alcohol problems in at least one other first degree relative. We will use a logistic regression model to compare the proportions of subjects with family history of such problems between the groups with one or two copies of the Asp40 allele and those homozygous for the Asn40 allele. Finally we will consider main effect differences between subjects with one or two copies of the Asp40 allele on the primary outcome measures as well as secondary measures.
Candidate gene and whole genome associations. The data from this trial will be combined with data from two open label studies of naltrexone to consider alternative genetic markers of treatment response as well as alternative endophenotypes. This work will be conducted principally by David Goldman, MD at NIAAA. In addition to treatment response, other endophenotypes to be considered are craving, intoxication, subjective high assessments, and alcohol effects. Given the continuous distribution of these measures, we would define upper and lower quartiles on each measure and examine associations using Dr. Goldman's addiction SNP marker chip. Candidate genes mentioned in the preliminary analysis section will also be considered for each of these endophenotypes. Finally, we will also consider an analysis of haplotype blocks for the primary hypothesis test. This essential becomes a sensitivity analysis for the A118G allele to determine if the effect is principally related to this single SNP.

D. Power analyses:
We anticipate being able to recruit 150 Asp40 subjects during the course of the funding period. Since it will be easier to recruit Asn40 subjects, we have planned to recruit as many Asn40 subjects as are required to obtain 80% power to detect the anticipated effect size for our primary hypothesis at a 5% significance level. As described below, this leads to a sample of 150 Asp40 and 190 Asn40 subjects.

In determining the sample size for this trial, we relied on the experience from our pilot study as well as past clinical trials to estimate the anticipated effect sizes. In this study, after accounting for adherence we demonstrated that 75% of subjects with one or two copies of the Asp40 variant had a positive response to treatment versus 52% homozygous for the Asn40 variant. Placebo response rates in our pilot study of highly adherent subjects were 44% and 39% in the two groups. We expect these response rates to be an upper limit of response based on selecting only those subjects who were highly adherent to treatment; we estimate the actual response rate to be in the range of 40-50% in NTX subjects homozygous for the Asn40 allele and 30-40% in the two placebo groups. However, it is noted that preliminary results from the COMBINE study suggest even larger effect sizes. For purposes of this application, we have estimated a 70-75% response in the NTX/Asp40 group. Moreover, based on prior experience we assume missing data (dropout) will occur in 15% of the randomized sample, with an additional 5-10% of subjects taking <50% of the prescribed medication, for a total estimate of 25% non-adherence. To estimate power for the primary hypothesis, we combine the methods of Fleiss for interaction tests in a binomial model, with the designs effect adjustments of Neuhaus and Segal to allow for the repeated measures within subjects, and the methods of Raudenbush and Liu to allow for possible variation in the interaction effect across the four sites [148-150]. For the analysis, we will consider relapse over the 12 one-week periods. We assume a 25% loss of subjects to dropout and non-adherence, and a compound symmetry structure for within-subject correlations, with conditional probability of 0.6 for use in one week, given use in the previous week. For success rates of 73% for the Asp40/NTX group and 46% for the Asn40 homozygous group/NTX group, and 35% for the two placebo groups, a sample of 135 Asp40s
and 170 Asn40s provides 80% power for detection of a significant interaction. This effect size corresponds to a naltrexone by polymorphism odds ratio of 3.17.

There is little prior information on the likely variation in effect across clinics. Because the populations in the different sites are very similar on measures of severity and on demographics (with the exception of gender, which is not expected to influence treatment effect), and because the procedures for the trial will be carefully controlled to ensure homogeneity across the four sites, we assume a small variation, with an effect size of 0.2. This increases the required sample size by about 10%, to 150 Asp40 and 190 Asn40 subjects. If we assume that there is no variation across sites, then the smallest detectable effect size drops from 3.17 to about 3.00.
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