# A Randomized Trial of Pioglitazone for the Treatment of Alcohol Use Disorder
# Protocol Version 1.5
# 2021-03-12

## 1. Background and Significance

1.1 Alcohol use disorders in VA: Alcohol use disorder (AUD) and heavy drinking are common among Veterans with 42.2% of Veterans having a life-time history of AUD and 14.8% screening positive for past-year probably AUD (1). AUD is associated with increased rates of accidents (2), domestic violence (3), neurocognitive impairments(4), poor medication adherence (5), increased mortality (6) and alcohol problems influence the course and treatment of many psychiatric and medical conditions (7, 8). Harmful alcohol use is common among Vietnam era Veterans (9, 10) and among returnees from Iraq and Afghanistan high rates of new onset heavy weekly drinking, binge drinking, and alcohol-related problems are reported (11). AUD is also a high cost disease within the VA. In Fiscal Year 2009, 334,130 Veterans had at least one VA encounter for an AUD. In a random sample of nearly 600,000 VA patients, Yu and colleagues found that the marginal yearly treatment cost (cost above the mean for the entire sample) associated with a diagnosis of an AUD was $3,124 per patient (12). This marginal cost multiplied by the number of Veterans with the disorder represents over 1 billion dollars in VA costs related to alcohol use disorders per year. Although treatments for AUD have improved over the past several decades (13), more effective interventions are needed. Pharmacological treatments for AUD are infrequently used in Veterans (14) and used less often than psychosocial interventions (15). However, without pharmacological adjunct to psychosocial therapy nearly 75% of patients resume alcohol use within one year (16). **Current pharmacological treatments are only modestly effective, at best; new medications that address AUD are needed.**

1.2 Medications for the treatment of AUD: Current medications for AUD target various brain regions that are classically thought to be involved in substance abuse behavior. Some of these regions include the nucleus accumbens and ventral striatum, thought to be involved in reinforcement, the dorsal striatum involved in habit formation, the hippocampus for its role in processing drug and alcohol contexts, and the prefrontal cortex for its role in exerting executive control over these regions. Most research has focused on GABA, glutamate and dopamine receptors as pharmacological targets as the above brain regions are mostly connected by GABAergic, glutamatergic and dopaminergic projections. In addition, compounds targeting opiate receptors have also been intensively studied (17, 18). The relationship between dopamine/dopamine pathways and craving/ reward has led to the clinical development of drugs that target this neurotransmitter in alcohol dependence (19). The Food and Drug Administration (FDA) has approved several medications for alcohol use disorder: disulfiram, an aldehyde dehydrogenase blocker; naltrexone, an opioid antagonist; injectable naltrexone (long-acting) and acamprosate, a functional glutamate antagonist. A meta-analysis of disulfiram showed limited efficacy and no effect on alcohol craving (20). Naltrexone has been shown to primarily reduce drinking severity rather than promote abstinence or non-heavy drinking (21). Early studies of naltrexone found the effect size of naltrexone was modestly higher than placebo but its clinical success for promoting abstinence and reducing heavy drinking has declined since the early single site studies (22). In clinical trials people treated with naltrexone had higher rates of dizziness, nausea and vomiting (23). Finally, oral naltrexone is contraindicated in acute hepatitis, liver failure, current opioid use, or anticipated need for opioids (24). Acamprosate has been FDA approved for AUD since 2004 and is primarily used to maintain abstinence, especially after alcohol detoxification (25). The treatment effect size of acamprosate is moderate in magnitude (25) and is limited by three times per day dosing and side effects which include anxiety, diarrhea and vomiting and is contraindicated in those with severe renal impairment (23).

Another drug that has shown efficacy in AUD studies is topiramate. Topiramate is FDA-approved for use as an anticonvulsant and as a drug to prevent migraine headaches. Its proposed mechanism of action for reducing alcohol use involves dopamine and dopamine pathways responsible for craving/reward in AUD by antagonizing excitatory glutamate receptors and inhibiting dopamine release while enhancing inhibitory GABA pathways (26). An initial study reported moderate to high effect size for reducing heavy drinking and for improving abstinence (27) however a meta-analysis using seven RCT's supported only a small to moderate effect size for topiramate (28). Also, adverse effects in those treated with topiramate are common and include cognitive dysfunction, paresthesias and taste abnormalities therefore drop-outs significantly impact its efficacy (23). Finally, gabapentin and baclofen are thought to act via GABAergic pathways. Most controlled trials of gabapentin have been small and of brief duration and none have included Veterans. Anton and colleagues added gabapentin to naltrexone during the first 6-weeks of a randomized controlled trial and found that the combination improved sleep and drinking outcomes as compared with naltrexone alone (and placebo) but the effect did not endure after gabapentin was discontinued (29). Furieri and colleagues showed that gabapentin reduced alcohol craving and alcohol consumption when compared to placebo; however, the study lasted only 28 days, used low dose (300mg twice per day) gabapentin and only included 60 total subjects (30). In a dose-ranging trial, Mason and colleagues found a linear-dose response with 1800mg of gabapentin being most effective in rates of complete abstinence and no-heavy drinking over a 12-week study (31). Side effects included fatigue (23%), insomnia (18%) and headache (14%) and only 56% of the subjects completed the study which suggests a significant limitation of this medication. Baclofen, another GABAergic medication has shown mixed results. An early study by Addolorato found significant improvement in alcohol outcomes in patients with cirrhosis but the study was relatively small (32) and later studies including our CSR&D funded study did not differentiate baclofen from placebo (33, 34). Finally, one important limitation of most of these medications is that they do not influence important alcohol outcomes that are associated with improved health. Specifically, in 2015 the FDA provided guidance for the development of drugs to treat "alcoholism" and recommended using responder analyses based on definitions that predict clinical benefit rather than analyses of group means as mean differences are difficult to interpret in regard to clinical relevance. They recommend using complete abstinence and no heavy drinking for defined periods of time, as efficacy endpoints (35). A variety of data suggest that the outcome of no heavy drinking is associated with reduced alcohol consequences, reduced likelihood of meeting future criteria for AUD, reduced risk of relapse and lower rates of alcohol-related problems (35). Most AUD medication studies have not shown this level of evidence in support of their use.

**In summary, these data suggest that medications to treat AUD have only small to moderate effect sizes; they are used infrequently and have limited impact on meaningful clinical outcomes that are associated with improved health. Thus, new medications with unique mechanisms of action and with improved tolerability and efficacy are needed to address AUD in Veterans.**

1.3 Relevance to Veterans Health: Veterans have high rates of AUD with significant impact on health, quality of life and mortality. In addition, the direct and indirect cost of AUD are high. Current medication treatment approaches are infrequently used and of only small to modest benefit. Pioglitazone has shown promise in several pre-clinical studies but no AUD clinically focused studies are available. If pioglitazone is found to be useful in reducing or eliminating alcohol use in Veterans it could be easily and rapidly repurposed to treat AUD, as it is already an FDA approved medication. Pioglitazone, given its unique mechanism of action, may offer an innovative approach to treating Veterans with AUD and thus help reduce the impact of this costly and difficult problem.

1.4 Pioglitazone a novel treatment for AUD: Peroxisome proliferator-activated receptors (PPAR) are a group of nuclear receptor proteins that primarily regulate gene expression via their role as ligand-activated transcription factors. PPAR agonists have been shown to reduce addictive behaviors in pre-clinical models including reducing chronic alcohol intake, binge alcohol intake, stress induced relapse and withdrawal in alcohol preferring rats (36). In addition, PPAR agonists have been shown to reduce a variety of behaviors related to other drugs of abuse, including nicotine, cocaine and heroin (37). There have been three PPAR isoforms identified, alpha, delta and gamma, each transcribed from different genes. Pioglitazone is a PPAR ɣ agonist and has been reported to decrease voluntary alcohol consumption of a 10% alcohol solution in rats genetically selected for high alcohol consumption. In addition, when rats had to perform an operant task to receive alcohol, pioglitazone reduced alcohol self-administration but not saccharin intake. These data suggest that pioglitazone reduces the motivation to consume alcohol (38).

Peroxisome proliferator-activated receptor agonists may exert their effects on alcohol use in a variety of ways. Activation of PPAR ɣ has been shown to mediate neuroprotection from N-methyl-D-aspartate (NMDA) mediated excitotoxic processes and inflammatory damage (37). PPAR ɣ agonists also inhibit pro-inflammatory IL-1 beta, IL-6, and TNF-α production and may exert its effects via reduction in innate immune signaling (39). Peroxisome proliferator-activated receptors have been found to be expressed in neurons, oligodendrocytes, and astrocytes in the central nervous system (cite). PPAR ɣ are highly expressed in the lateral hypothalamus (LH), the paraventricular nucleus of the hypothalamus (PVN), the arcuate nucleus (ARC), and the ventral tegmental area (VTA). PPAR ɣ receptors co-localize with tyrosine hydroxylase in the VTA suggesting the expression of this receptor in dopaminergic cells and thus pioglitazone may reduce this rewarding/reinforcing effect in AUD (40-42). Although, several pre-clinical studies suggest a significant positive effect from PPAR agonists on alcohol and drug use, only one human study is available and that was focused on treating cocaine use disorder (43). The 12-week pilot study randomized 31 patients with cocaine use disorder to either pioglitazone or placebo and found a higher likelihood of reduced cocaine craving in patients treated with pioglitazone. Adherence to pioglitazone was high and side effects were mild, no serious adverse events occurred. Twenty-six subjects also met criteria for AUD and the data suggest that pioglitazone was effective at reducing alcohol use over placebo (43).

1.5 Summary of Pioglitazone pharmacological and toxicological data: Pioglitazone was FDA approved in 1999 to improve glycemic control in patients with type 2 diabetes, thus side effects and risks are well known. The FDA package insert provides information on a variety of risks and potential side effects that is summarized: The most common side effects (>5%) include upper respiratory tract infection, pharyngitis, headache, sinusitis and myalgia. Although the overall risks of these problems are greater than 5%, the risks when compared to placebo are all less than 5%. Of note, there have also been several warnings issued regarding pioglitazone. First, pioglitazone alone or more commonly in combination with other antidiabetic agents (typically insulin) can cause fluid retention and edema which increases the risk of congestive heart failure. The risk of heart failure was more common when comparing pioglitazone to placebo in a high-risk group of patients with diabetes and pre-existing cardiovascular disease (44). There have been several reports of hepatotoxicity but not enough data to establish probable causality. There was a small increased rate of fractures in women and a small increased risk of urinary bladder cancer in patients treated for 24 months or longer. Finally, pioglitazone is a category C medication and thus should not be used in pregnancy and it may also induce ovulation so individuals may be at greater risk for pregnancy when taking this medication (45).

In addition to the package insert there are a variety of published data in a diverse number of conditions including four published reports in patients treated for depression and a recent study that used pioglitazone to treat cocaine use which included 26 individuals with alcohol use disorder- see above. Studies with important safety information are summarized here: A meta-analysis of pioglitazone used for reducing liver fibrosis in nonalcoholic steatohepatitis reviewed 8 RCT's with 516 patients followed between 6 and 24 months and found weight gain and lower limb edema as the primary adverse events (46). A recently published article using pioglitazone, for 30 months, in a similar clinical population, found no difference of major drug-related adverse events with minimal weight gain being the primary difference between pioglitazone and placebo (47). Although prior retrospective studies led to the FDA boxed warning for bladder cancer, two recent prospective studies suggest no link between pioglitazone and bladder cancer (48, 49). There have been four published RCT's of pioglitazone for major depressive disorder with a total of 161 patients enrolled (6-12 week studies) with no significant adverse events occurring (50-53). Pioglitazone has also been explored as a treatment for dementia. A recent meta-analysis using PPAR- γ agonists to treat dementia found 9 studies in 4,327 participants and found no significant difference in adverse or serious adverse events (54). A recent placebo controlled trial of pioglitazone for 30 patients with cocaine use disorder of whom 26 had an AUD reported the most frequent side effects were sleep disruption, diarrhea, stomach pain, cough, and increased urination which were all rated as mild (43). Finally, a recent, very small randomized trial comparing pioglitazone to placebo in a "mechanistic proof or principle" study to determine the effect of pioglitazone on alcohol craving was stopped early after 16 subjects were randomized due to safety concerns. The study entailed two experimental manipulations designed to induce alcohol craving, guided imagery compared to intravenous lipopolysaccharide. A total of 14 subjects (6 pioglitazone and 8 placebo) were analyzed. Five in the pioglitazone arm had elevated creatine kinase (CK), one was deemed serious and one receiving placebo had an elevated CK. The study did not report absolute values for CK nor whether or how the CK was related to the initiation of pioglitazone or other whether it was temporally related to other study procedures (Schwandt et al psychopharmacology 2020). **In summary, pioglitazone is well tolerated with few side effects or significant safety concerns, particularly in short term (less than 24 weeks) studies.**

**2.0 Preliminary Studies**: The Minneapolis and Long Beach VAHCS have a long history of scientific collaboration. We have completed two prior CSR&D funded randomized controlled trials in AUD. The first was a RCT of motivational enhancement therapy in patients with AUD and chronic hepatitis C. We were able to show that motivational enhancement therapy helped reduce alcohol use in this population (55). The second was a placebo controlled RCT of baclofen in patients with AUD and hepatitis C. This was the largest trial of baclofen and showed no effect of baclofen on reducing alcohol use in this population (34). As previously noted there are no published trials of pioglitazone focused on AUD. However, in a preliminary investigation at the Minneapolis VA, we identified 252 patients who had at least one prescription for pioglitazone during 2016, all of these patients were being treated for diabetes. Of these 252 patients 51 had an AUDIT-C score of at least 3 prior to starting the pioglitazone. The average AUDIT-C score prior to starting pioglitazone was 4.18 (1.69) the average AUDIT-C score after Pioglitazone was started was 2.85 (1.76). Patients stopped and started pioglitazone at various times and we determined the effect of pioglitazone on AUDIT-C scores by using a mixed regression model to compare AUDIT-C scores while on pioglitazone with those scores when not on pioglitazone. This analysis suggested that pioglitazone was associated with a reduction in AUDIT-C scores ($F(1,312.2)=14.29$, $p<.001$). **These data, combined with preclinical studies and the Schmitz study of cocaine using patients, suggest that pioglitazone is an effective**

**medication for AUD. A well-designed trial of pioglitazone focused on AUD is warranted and needed.**

## 3.0 Research Design and Methods

### 3.1 Overview

**This proposed research study is a double-blind controlled clinical trial of 200 Veterans with AUD randomized to either pioglitazone or placebo.**

After screening visits and informed consent, participants who meet all inclusion and exclusion criteria and who sign the informed consent will be given a breathalyzer test and test result must be below 0.05 in order for potential participant to be enrolled (see Breathalyzer Testing section, precautions will be taken based on test result). Following the breathalyzer test, participants will complete the initial assessment instruments, which include the SCID, Obsessive Compulsive Drinking Scale (OCDS), Timeline Follow Back (TLFB), Beck Depression Inventory-2nd edition (BDI-II) and the PTSD Checklist (PCL-5). They will also provide a urine sample for a urine drug screen, Ethyl Glucuronide (EtG), and Ethyl Sulfate (EtS), and blood samples for ALT, AST and BNP. Women of childbearing potential will provide a urine sample for Beta-Human chorionic gonadotropin (β-HCG). Participants will then be randomized to receive either pioglitazone or a placebo (see **Data Analysis Plan-Randomization** section).

After randomization, all the participants will be seen weekly for the first 4 weeks (visits 1,2,3,4- baseline or randomization visit will be visit 0) then every 2 weeks until the end of the study (week 6 or visit 5, week 8 or visit 6, week 10 or visit 7, week 12 or visit 8, and week 14 or visit 9) for a maximum of 12 visits (including the screening visit, baseline visit, and closeout visit). At week 16, there will be a termination or closeout visit after study medications have been tapered. All study measures will be administered at each visit during the double-blind period from weeks 0 to 14 and week 16 or the closeout visit. In particular, the BDI-II and PCL-5, will be given at each study visit. If participants report significant depressive or other psychiatric symptoms during the study, the decision to exclude them will be based on whether they can safely participate in the remainder of the study (See **Human Subjects** section). Data will also be collected from participants' medical records regarding enrollment and attendance at specialty alcohol treatment appointments. Follow-up assessments will be conducted in person by a rater blinded to the participant's study condition.

The initial screening and baseline visit (may be done at the same time) and week 4 and 14 visits will be conducted in person. However, to enhance subject safety and minimize the risk of COVID-19, all other visits will be conducted by phone but may be conducted in person if the patient requests or if in the opinion of the PI a face-to-face visit is necessary for subject safety.

Participants will have medication adherence assessed at each study visit (total number of tablets dispensed – tablets reported taken divided by the total number of tablets dispensed taking into consideration that patients will be given additional study medication in the event of missed visits- 7 days of additional medication). Tablet counts will be confirmed at each of the face-to-face visits. Participants will have a breathalyzer test at each face-to-face visit and will not be allowed to complete assessments if their test result is above 0.05%. For phone visits, subjects will be evaluated clinically for intoxication ie coherent speech, slurred words, able to follow questions and respond appropriately.

| | **May occur at same visit** | | **Double Blind Period** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Week** / **Measure** | **Screening** | **Baseline** | **Week 1** | **Week 2** | **Week 3** | **Week 4** | **Week 6** | **Week 8** | **Week 10** | **Week 12** | **Week 14** | **Week 16** |
| Informed Consent | X | X | | | | | | | | | | |
| Eligibility Determination | X | | | | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SCID DSM-5 | X | | | | | | | | | | | |
| Randomization (pioglitazone or placebo) | | X | | | | | | | | | | |
| Demographics/ Medical History | X | | | | | | | | | | | |
| Pharmacy Dispenses Study Drug (pioglitazone or placebo) | | X | X | X | X | X | X | X | X | X | X | |
| Determination of Study Drug Compliance | | | X | X | X | X | X | X | X | X | X | X |
| BBCET | | X | X | X | X | X | X | X | X | X | X | |
| Breathalyzer | X | X | X | X | X | X | X | X | X | X | X | X |
| BSI | | X | X | X | X | X | X | X | X | X | X | X |
| BDI-II | | X | X | X | X | X | X | X | X | X | X | X |
| PCL-5 | | X | X | X | X | X | X | X | X | X | X | X |
| OCDS | | X | X | X | X | X | X | X | X | X | X | X |
| TLFB | X | X | X | X | X | X | X | X | X | X | X | X |
| EtG/EtS, (Urine) | | X | | | | X | | | | | X | |
| ALT, AST, GGTP, BNP, CK | | X | | | | X | | | | | X | |
| CRP | | X | | | | X | | | | | X | |
| Inflammatory markers | | X | | | | X | | | | | X | |
| Medication side effects | | | X | X | X | X | X | X | X | X | X | X |
| Attendance at EtOH Tx | X | | X | X | X | X | X | X | X | X | X | X |
| Urine Drug Screen, β-HCG | X | | | | | | | | | | | |

Participants will provide a urine sample for EtG, EtS, and β-HCG (for women of childbearing potential), ALT, AST, GGTP, CK and BNP at baseline. The EtG, EtS, ALT, AST, GGTP and BNP, will be repeated at weeks 4, and 14.

3.2 Participants: Male and female Veterans above the age of 18 will be recruited from outpatient clinics at the Minneapolis and Long Beach VAHCS's and through advertisements at each site.
3.3 Screening: The AUDIT-C will be used as a method to identify potential participants. The AUDIT-C is used clinically to screen for and identify heavy alcohol use in all VA's. Patients with an elevated AUDIT-C of 4 or greater will be contacted by study staff via letter and recruited to the study. Clinical staff can also refer patients with a similar AUDIT-C or if there is concern for heavy alcohol use.

Individuals who are interested in the study will asked if they would like to participate in a qualifying assessment. If apparently qualified and interested in participating in the study, they will be given the consent form to read by a study staff member. The study staff member will answer their questions and determine whether the individual understands the study through a series of pre-determined questions. The questions will include whether the individual understands the key tasks, risks, benefits of participating in the study as described in the consent form. If the individual understands these details and chooses to sign the consent form, they will participate in the qualifying assessment. This assessment is designed to determine if the participant fully meets the inclusion and exclusion criteria for participation in the study. The results of the assessment are reviewed by the PI to make a final determination of eligibility for the study's clinical trial. If the patient does not qualify this will be explained to the patient, they

will be paid for the qualifying assessment and given referral information if deemed appropriate by the PI.

3.4 Inclusion criteria:

1) DSM-5 diagnosis of at least moderate alcohol use disorder using the SCID
2) A mean of six heavy drinking days per month for the 3-months prior to baseline.
3) Drinking at least 14 drinks for men or 7 drinks for women, or more per week for the 4 weeks preceding the screening visit.
4) Willingness to provide contact information to confirm study follow-up appointments
5) Ability to perform informed consent
6) Female subjects: a negative pregnancy test
7) Serum ALT < 3 times reference range
8) Stable psychiatric medication doses the month prior to baseline visit (antidepressant, antipsychotic, subjects may have changes in trazodone for sleep)

3.5 Exclusion criteria:

1) Current DSM-5 diagnosis of moderate to severe psychoactive substance use disorder (i.e. cocaine, opiates, methamphetamine) other than cannabis or nicotine
2) Medical conditions contraindicating pioglitazone pharmacotherapy (e.g., congestive heart failure, clinically significant edema, clinically significant liver disease, hypoglycemia, diabetes, history of bladder cancer)
3) Taking medications known to have significant drug interactions with the study medication (CYP2C8 inhibitors or inducers, antihyperglycemic medications)
4) Cognitive or physical impairment that precludes study participation
5) Currently and seriously suicidal (i.e., plan and intent)
6) Currently being treated for AUD with a medication (naltrexone, naltrexone injectable, acamprosate, topiramate, disulfiram and gabapentin)
7) Impending incarceration
8) Pregnant or planning to become pregnant during the course of the trial or nursing for female patients
9) Unwillingness to sign a written informed consent form
10) Unwillingness to use a barrier method of birth control during the study for female patients

3.6 Feasibility

Currently, the Minneapolis VAHCS serves approximately 100,000 unique Veterans and 9,550 were diagnosed with an AUD. At the Long Beach VAHCS approximately 60,000 unique Veterans are seen each year and nearly 5,425 received a diagnosis of AUD. Furthermore, each site has a long history of research of AUD and a strong collaboration between researchers and clinical staff at each site. We have also collaborated on several similar studies and have experience in recruiting and maintaining participants with similar characteristics from our work on two prior CSR&D funded RCT's, "Efficacy & safety of baclofen to reduce alcohol use in veterans with HCV" and "Motivating Chronic Hepatitis C patients to Reduce Alcohol Use". In these studies we successfully recruited 180 subjects with AUD and hepatitis C (a much smaller subgroup of all Veterans with AUD) during the course of the studies. Thus, we expect to be able to easily meet our recruitment goal of 200 subjects during the 4 year study. Finally, we have

already received an Investigational New Drug exemption for pioglitazone (see attachment) so this will not be a barrier to starting the study.

3.7 Intervention

At the baseline (week 0) visit participants will be randomized to receive either pioglitazone or a matching placebo by the research pharmacist (**see Randomization section**). Participants and study personnel will be unaware of the treatment assignment. In order to maintain study blind but insure patient safety, sealed envelopes that contain the identification of the study medication (pioglitazone or placebo) will be kept in the research pharmacy and opened only in case of emergency. Study medication will be dispensed in a double-blind fashion during the entire study period. Study medication will be dispensed at baseline and weeks 1,2 and 3 sufficient for a 2-week period (taking into consideration the possibility of missed visits) and will be given at weeks 4,6,8,10, and 12 sufficient for a 3-week period (taking into consideration the possibility of missed visits). Placebo capsules will be of an identical size, shape and color as pioglitazone. Active medication will be started at 15mg per day and titrated over two weeks until reaching a target dose of 45mg per day. This is the FDA maximum dose and was selected based on a prior small study focused on the treatment of cocaine use (43).

All participants will receive Brief Behavioral Compliance Enhancement Treatment (BBCET) as their psychosocial treatment. This is a standardized 15-minute intervention that emphasizes medication adherence as a crucial element to change alcohol use behavior (56). It will be administered at every visit. BBCET has been used in previous AUD medication trials as the psychosocial treatment (27, 57). We also used the BBCET for the CSR&D funded "Efficacy & safety of baclofen to reduce alcohol use in veterans with HCV" and have extensive knowledge in delivering this intervention (34). Study staff will be trained to administer the BBCET and 2% of sessions will be observed for adherence to the intervention.

3.8 Compensation

Participants will be compensated $20 for completion of study questionnaires and providing urine and blood samples at the baseline visit and $20 for completing each follow-up visit. If participants complete the data collection at all 11 time points they will receive a total of $220.

3.9 Measures

Screen for current intoxication. Blood alcohol concentration (BAC) will be measured using the AlcoMate Revo Breathalyzer which measures BAC% between 0.00% and 0.400% in 0.01% steps. Sensor accuracy of the AlcoMate is +/- 0.02% BAC. This measure will be taken prior to any study related interactions with subjects to insure that they are not intoxicated at the time of evaluation. If a subject's BAC is above 0.04% scheduled tasks for that day will be rescheduled. If the subject is driving, alternative arrangements will be made for their transportation home.

3.9.1 Rating Scales

The Structured Clinical Interview for DSM-5 (SCID). The SCID will be used at the initial assessment to identify alcohol use disorder and other substance use disorder (58). All SCID interviewers will be trained by a qualified SCID trainer (including PI and CO-I's) and will

demonstrate competency in administering the SCID. Research has shown that this method for determining psychiatric diagnostic information is reliable and valid (59).

The Time Line Follow-Back (TLFB). The TLFB interview will be used to identify daily drinking patterns of subjects in the three months before treatment and the intervals between study assessments (60). The TLFB has been shown to have adequate psychometric qualities in both clinical and research populations (61). Using a calendar, the respondent provides retrospective estimates of daily alcohol drinking over a specified amount of time. All primary and secondary outcomes will be derived from the TLFB assessment including: 1) heavy drinking over the last 8 weeks of the study; 2) percent or number (depending on the distribution) days drinking per month; 3) number of drinks per drinking day; and 4) number of heavy drinking days per month (heavy drinking days defined as for men: >4 drinks in a day or women: >3 drinks in a day).

Beck Depression Inventory-2nd edition. The BDI-II is a 21-item self-report instrument that will be used to assess depressive symptomatology. Total score of 0-13 is considered minimal range, 14-19 is mild, 20-28 is moderate and 29-63 is severe symptoms. The BDI-II has been shown to have good validity and reliability in various psychiatric populations (62). The BDI-II will be administered at the initial and all follow-up assessments. The suicide ideation item will be reviewed at every visit prior to completion of visit and if positive will prompt a suicide risk assessment and further evaluation and/or referral for treatment.

Brief Symptom Inventory (BSI). The BSI is a 53-item self-report measure that will be used to assess several dimensions of psychopathology. The scale has nine primary symptom dimensions and three global indices. It is a reliable and valid instrument and has been normed to both adult non-patients as well as adult psychiatric patients (63). It will be administered at baseline and all follow-up assessments.

PTSD Checklist for DSM-5 (PCL-5). The PCL-5 is a brief 20-item self-report instrument that will be used to assess Post Traumatic Stress Disorder symptoms. Total score of 0-33 is considered not indicative of severe symptoms of PTSD, 34-80 is considered indicative of symptoms of PTSD. The PCL-5 has been shown to have good validity and reliability in various populations (64). The PCL-5 will be administered at the initial and all follow-up visits.

Medication Side-effect checklist: We will develop a side effect checklist to monitor any adverse effects from the medication. We will base the checklist on the package insert and data from prior studies, including the Schmitz et al study.

3.9.2 Biological measures of Alcohol and other substance use

Urine drug screens (UDS). Participants will be asked to provide a urine sample for UDS at the baseline visit. The results will be used as an objective measure of recent drug use to supplement self-reported measures. Results will be issued as either positive or negative for a substance; no quantification of the concentration of the substance in the urine will be given. UDS will be obtained at baseline and at all follow up assessments.

Ethyl Glucuronide (EtG). Participants will be asked to provide a urine sample to assess EtG at baseline, 4, 8 and 12 weeks. Results will be correlated with self-report measures of alcohol use and GGT in order to test the hypothesis that changes in alcohol consumption and GGT will be reflected by changes in EtG. EtG is a direct metabolite of alcohol; it remains in urine

for up to 5 days after cessation from alcohol, is highly sensitive and has good specificity for alcohol use (65).

Ethyl Sulfate (EtS). Participants will be asked to provide a urine sample to assess EtS at baseline, 4, 8 and 12 weeks. EtS is a direct metabolite of alcohol; it remains in urine for up to 5 days after cessation from alcohol, is highly sensitive and has good specificity for alcohol use (65). Additionally, we are already collecting urine for EtG and the laboratory that tests for EtG is already combining the EtS with EtG at no additional cost.

Gamma-glutamyl transferase (GGT): is an enzyme that transfers gamma-glutamyl functional groups. It is found in many tissues, the most notable the liver, and has significance in medicine as a diagnostic marker of alcohol use (66).

Alanine Aminotransferase (ALT): is an enzyme that converts alanine into pyruvate. It is mainly found in the liver and be increased with liver injury.

Aspartate Aminotransferase (AST): is a pyridoxal phosphate-dependent transaminase enzyme that catalyzes the reversible transfer of an α-amino group between aspartate and glutamate. It is mainly found in the liver and may be increased with liver injury. An AST to ALT ration of 2:1 is suggestive of alcohol related liver injury (66).

Creatine Kinase (CK): is an enzyme expressed in various tissues and as a blood test is a marker of muscle injury.

3.9.3 Laboratory Safety Measure

B-type natriuretic peptide (BNP): is a hormone secreted by cardiomyocytes in response to stretching caused by increased ventricular blood volume. A plasma BNP (≤ 100 ng/L) provides an excellent ability to exclude heart failure with high sensitivity (67).

3.9.4 Inflammatory Markers

C-Reactive protein (CRP): is a protein found in the blood plasma that rises in response to inflammation. CPR will be assessed to check inflammatory responses to pioglitazone. Other inflammatory markers such as interleukin-6,8 and 12 as well as tumor necrosis factor- alpha and SB100 a marker of blood brain barrier inflammation will be tested. One of pioglitazone's purported mechanism of action is via inflammation thus we will test for these markers.

3.10 Potential hazards to personnel and precautions: Collection of blood and urine samples may pose a hazard to study personnel. Contact with blood or urine has the potential to transmit disease. The collection of blood will be performed in the existing Minneapolis and Long Beach VAHCS laboratories where lab technicians are already trained and utilize universal precautions. The collection of urine samples will be completed in the clinics by research staff. All staff will be trained in universal precautions and the study will comply with all safety regulations and training stipulated by VA.

3.11 Follow-up: At each visit, research coordinators will administer all measures, dispense medication and deliver the BBCET. Any concerning side effects or lab abnormalities will be reviewed with the PI or site PI. Appropriate medical evaluation will be undertaken as deemed necessary by the PI's. Subjects who miss three consecutive visits will stop the medication as they will have been off of medication for minimum of 1 week. Any serious adverse side effects where the blind needs to be broken will result in stopping the assigned treatment. However, every effort will be made to gather research data through the end of the study.

3.12 *Randomization:* Participants will be randomly assigned to one of two groups (pioglitazone or control/ placebo) using a minimization method of Pocock and Simon (1975)(68). Randomization will be stratified by site (Minneapolis and Long Beach) and will be handled centrally by the Data Coordinating Center at the Minneapolis VA, and a special randomization program will be developed in consultation with a statistician in Minneapolis (Dr. Paul Thuras). The program will track the balance of stratification variables (i.e., site) for each condition and change the probability of assignment if the variables are unbalanced. This method assures that the two groups are comparable with respect to the stratification variables throughout the study period.

Each site will be provided a number of envelopes with randomization results sealed inside. Privacy envelopes will ensure that the results cannot be detected without breaking the seal. The envelopes will be numbered and will be opened in that order. The number of envelopes will be the number of expected enrollees plus 10 extra in order to account for non-compliant participants and dropouts. These envelopes will only be opened by the research pharmacist. This procedure will be used to maintain the blind for the PI, research assistants, study coordinators and therapists. Only the research pharmacy will be allowed to know the randomization for each participant. The pharmacist will keep all envelopes containing the randomizations. When a subject signs the informed consent form, the pharmacist will be called and asked to select the next envelope and open it, fill out the participant number and dispense study medication according to the randomization. If a physician or medical professional needs to know whether the patient is taking the study drug, a wallet card given to the participant will contain the number of the research pharmacy. The professional can call the pharmacy to access the list of participants and can break the blind for that participant.

## Statistical Considerations

### 4.0 Data Analysis Plan

4.1 Data Management.

Study data will be collected and managed using VA REDCap (Research Electronic Data Capture) hosted behind the VA firewall on a VINCI server. REDCap is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

4.2 Data quality/integrity.

All data will be obtained by an assessor (the research coordinator) who will be blinded to the treatment condition. All data will be inspected for missing and outlier values and response set patterns. Distributions will be graphically represented and inspected visually and quantitatively for departures from normality and other irregularities. All data will be characterized in terms of means, standard deviations, histograms, and response ranges for continuous variables and frequencies and counts for categorical variables. A random sample of 5% of

cases will have all hard copy data compared to entered data for accuracy, including all measures scored by computer algorithms. The need for data re-entry or recoding, data transformations and non-linear or non-parametric analyses will be based on these results.

4.3 Descriptive analyses

To confirm that the randomization scheme achieved the intended aim of evenly distributing important study characteristics, groups will be statistically compared on baseline demographic and clinical variables. Any significant group differences on these baseline variables will be factored into study analyses (e.g., using covariates).

4.4 Primary and secondary outcome measures

For the primary outcome we will use change in number of heavy drinking days per week. Secondary outcome measures will include a responder analysis, the rate of no heavy drinking over the last 8 weeks of the study, and another continuous measure of alcohol use including number of drinks per week over the entire study period. Heavy drinking days per week was chosen as it is a standard outcome used previously used in clinical AUD trials (34, 55). The responder analysis measure of no heavy drinking days over the last 8 weeks of the active study period (weeks 6-14) was chosen as a secondary outcome as it was recently recommended by the FDA in their guidance to industry for the development of drugs for alcoholism (35). The FDA report indicated that a responder analysis is preferable to analysis of group means as mean differences are difficult to interpret with regard to clinical relevance. In addition, changes in this measure is associated with good clinical outcomes (35). Each subject will be assessed for the outcome variables (alcohol consumption) at baseline (date of randomization), 1,2,3,4,6,8,10,12 and 14 weeks (week 16 is a safety check as medications will be tapered off starting at week 14). For the responder analysis, we will use Chi -square to test the difference between pioglitazone and placebo for the rate of no heavy drinking over the last 8 weeks (week 6-14) of the study. Hypotheses will also be tested across all time points to establish temporal patterns of group effects. For the continuous measures of alcohol use we will use mixed effects models if data residuals are normally distributed and generalized estimating equations (GEE) if the data are non-normally distributed (69, 70). Both are flexible regression methods for incomplete repeated measures data and allow continuous and categorical covariates, fixed and time-dependent covariates, and a specification of unstructured as well as structured covariance matrix. Secondary hypotheses will be evaluated using chi-square tests of proportions and logistic regression, with statistical significance criterion also set at $p < 0.05$ for categorical outcomes. Zero-inflated negative binomial analyses will be used to contrast the study groups on count variables if warranted by a high percentage of zero alcohol use days over the follow-up (71).

4.5 Power Estimate

The primary intention to treat population will include all participants randomized. The effect size of pioglitazone is estimated based on studies. Schmitz et al found a small effect size favoring pioglitazone (94% likelihood of decrease in alcohol use versus 83% in placebo) which is likely low as all the subjects studied had a primary cocaine use disorder diagnosis with an AUD secondary. Based on a sample of Veterans at the Minneapolis VAHCS who had taken

pioglitazone during fiscal year 2016 we found that taking pioglitazone was associated with decreased alcohol use as measured by the AUDIT-C with and effect size of d=.48.

Using G*power we estimated samples size for various base rates of drinking (heavy drinking days per week) by various differences in reductions in rate produced by pioglitazone versus placebo across the 14 weeks follow-up period using Poisson regression. Baseline heavy drinking days per week in our Baclofen study, was 3.5 per week. Based on this we estimate that we are adequately powered to find clinically meaningful differences in reduction (20% or greater) given our proposed sample size.

| Base Rate per week (Heavy Drinking Days) | Reduction in rate. | Power | Total N |
|---|---|---|---|
| 4 | 19% | 80% | 164 |
| 3 | 19% | 80% | 217 |
| 2 | 19% | 80% | 321 |
| | | | |
| 4 | 20% | 80% | 147 |
| 3 | 20% | 80% | 194 |
| 2 | 20% | 80% | 288 |
| | | | |
| 4 | 21% | 80% | 132 |
| 3 | 21% | 80% | 175 |
| 2 | 21% | 80% | 260 |
| | | | |
| 4 | 22% | 80% | 120 |
| 3 | 22% | 80% | 159 |
| 2 | 22% | 80% | 235 |
| | | | |
| 4 | 23% | 80% | 109 |
| 3 | 23% | 80% | 145 |
| 2 | 23% | 80% | 214 |

Thus, we feel that the sample size of 200 (100 per site) is still warranted.

For **secondary outcomes** including the categorical outcome (no heavy drinking days in the last 8 weeks of the study) with 90 patients in each group (100 recruited subjects in each group, assuming 20% attrition) we estimate that this sample size will allow us to find a 22% difference (OR=2.45) in heavy drinking days with 80% power at p<.05. Both mixed models and GEE allow us to take full advantage of the ITT sample and thus power is based on 90 subjects per group. Other secondary measures including biomarkers of alcohol use (EtG/EtS and GGT) and for craving we will use linear mixed models to compare change in biomarkers and change in OCDS scores. Estimates for comparisons of these continuously distributed outcomes indicate

that we will be able to find effect sizes of d=.47 with 72 subjects or larger with 100 subjects per group.

*4.6 Attrition: We* anticipate an attrition rate of 20% and this has been considered when calculating the budget and in the power analysis. Rationale for this estimate includes several factors. The attrition rate in our prior studies ranged from 14% (55) to 24% (34). Second, some patients may be more interested in remaining in a medication study with high perceived efficacy. The medication will be dispensed at the outpatient clinic, which should reduce the stigma typically associated with specialty alcohol treatment. Finally, participant compensation will provide an incentive to complete the follow-up sessions. However, we have reviewed other VA alcohol treatment studies that showed great variation in attrition rates, **therefore we are projecting a conservative estimate that 80% of the participants consented will complete the study.**

*4.7 Missing Data:* We will perform an intent-to-treat analysis, meaning that all subjects randomized into two treatment groups will be included in the analysis regardless of the extent of compliance with the treatment or withdrawals during the trial. This will create incomplete data because some subjects will withdraw from the study during the course of the treatment, thereby their responses will be considered missing after withdrawal**. If the dropout process is related to the outcome measures (i.e., alcohol use), this will present a challenge in the analysis.** The majority of the currently available statistical methods assume that data are missing at random. However, in this study, it is plausible that the likelihood of dropout is related to the level of or change in alcohol use. For example, those with increase or no reduction in alcohol use may be more likely to drop out of the study. When subjects drop out of the study, we will attempt to obtain data on reasons for dropout. We will analyze whether the dropout process is at random with respect to the outcome measures. If the dropout process is related to the outcome measures, we will utilize models that incorporate a nonrandom dropout mechanism (Little, 1995). These analyses will be carried out as the secondary analyses for hypothesis-generating purposes. Use of the EM algorithm in the analysis will allow inclusion of observations with missing datapoints. This increases power and improves the ability to produce estimates with less bias.

## 5.0 Summary

The proposed work seeks to improve alcohol outcomes in Veterans with an AUD and current alcohol use. Current medications for AUD are of only modest benefit and relapse and continued alcohol use are common. Preclinical data strongly suggests that pioglitazone will be helpful in reducing alcohol use in humans. This project will provide important data regarding the efficacy of pioglitazone. Should the results prove positive, we would seek funding for a larger multi-site study (potentially a cooperative study) to confirm the results. Finally, as pioglitazone is already an FDA approved medication it could easily be re-purposed and used immediately to help Veterans with AUD.

## 6.0 Project Timeline

| Months | Activities |
|---|---|
| 1 to 3 | 1) hire staff; 2) purchase supplies; 3) create study documents and database; 4) obtain pioglitazone and placebo |
| 4 to 12 | 1) recruit 44 participants or 5.5 subjects per month- this is 2-3 subjects per site and very feasible; 2) begin follow-up data collection; 3) begin data entry; 4) quarterly DSMB reports. |
| 13 to 24 | 1) recruit 64 participants; 2) follow-ups continues; 3) continue data entry; 4) quarterly DSMB reports |
| 25 to 36 | 1) recruit 64 participants; 2) follow-ups continues; 3) continue data entry; 4) quarterly DSMB reports |
| 37 to 42 | 1) recruit 28 participants; 2) follow-ups continues; 3) continue data entry; 4) quarterly DSMB reports |
| 43 to 48 | 1) recruitment complete follow-up data collection only; 2) clean data; 3) break blind; 4) begin data analysis<br>2) complete data analysis; 2) manuscript preparation and submission |