N-Acetylcysteine for Youth Alcohol Use Disorder

Study Protocol

NCT03707951

Most recent IRB-approved Protocol, dated July 11, 2023 (no updates since)

Submitted to ClinicalTrials.gov on December 30, 2024

1.0 Objectives / Specific Aims

Adolescence is a key period in the development of problematic alcohol use, with approximately 15% of 18-year-olds meeting criteria for alcohol use disorder (AUD). Efforts to prevent or diminish adolescent heavy alcohol use have been only modestly effective, with up to 86% of youth returning to use within 12 months following treatment. Inadequate treatment for adolescents is an important public health concern given that alcohol use during this time is highly predictive of long-term problematic drinking. Effective treatment during this vulnerable period could help prevent more severe, treatment-resistant AUD in adulthood. While several medications have demonstrated efficacy for adult AUD, minimal pharmacotherapy research has focused on adolescents. Evaluation of alternative and more efficacious treatments for adolescents with AUD is warranted.

Among candidate pharmacotherapies for adolescent AUD, the over-the-counter antioxidant supplement *N*-acetylcysteine (NAC) is particularly compelling. An easily accessible and affordable compound with established safety across age groups, NAC has shown promise in treating a variety of substance use disorders by restoring glutamate homeostasis disrupted by addiction. Our team has demonstrated, via a placebo-controlled randomized trial, that NAC promotes abstinence in adolescents with cannabis use disorder (CUD). Specific to alcohol, a number of recent preclinical studies have demonstrated superiority of NAC, compared to placebo, in reducing alcohol self-administration. These findings, coupled with findings of alcohol reductions in our team's NAC trials for CUD, indicate that a trial to directly assess the effects of NAC on adolescent AUD is warranted.

In addition to evaluating the effects of NAC, the proposed Phase II proof-of-concept study is designed to gather key data to inform future Phase III treatment efficacy trials for adolescent AUD, including effect size estimates. There is no currently established template for adolescent AUD pharmacotherapy trials. While adult trials may be informative, adolescents with AUD often differ from adults in alcohol use patterns, use-associated problems, and receptiveness to, as well as adherence with, treatment. Before proceeding to a Phase III efficacy trial in adolescents, a number of developmentally-informed methods must be embedded and evaluated for feasibility and value in a proof-of-concept trial. In addition to traditional, established, office-based clinical trial procedures, we propose utilizing mobile technology to improve data collection, engagement, and medication adherence. We additionally propose comparing the value of a cross-section of self-report and biological measures of alcohol use and related behaviors, to determine the most appropriate measures for inclusion as efficacy outcomes in Phase III trials.

With these methodological considerations in mind, and in response to National Institute on Alcohol Abuse and Alcoholism (NIAAA) RFA-AA-18-009 *Medications Development for the Treatment of Alcohol Use Disorder*, we propose preliminarily testing the effects of NAC, compared to placebo, on a platform of weekly evidence-based brief alcohol intervention for adolescents with AUD (*N*=120). We will (1) conduct an 8-week, intent-to-treat, double-blind, parallel-group, randomized, placebo-controlled trial of NAC (2400 mg per day); (2) examine standardized, repeated dependent measures of clinical outcomes at baseline, throughout treatment, and at post-treatment follow-up; and (3) employ mobile technology to improve data collection, engagement, and medication adherence. The following specific aims are proposed:

Specific Aim 1. To evaluate the effect of NAC, as compared to placebo, in reducing alcohol use (total standard drinks) among treatment-seeking adolescents with AUD.

Specific Aim 2. To evaluate the feasibility and utility of implementing developmentally-informed methods, including those that leverage mobile technology, to conduct a randomized, placebo-controlled trial of a pharmacotherapy for adolescent AUD.

Specific Aim 2a: Examine agreement between self-report (mobile technology-delivered daily self-report, Timeline Follow-Back) and biomarkers (ethyl glucuronide, carbohydrate deficient transferrin, phosphatidylethanol, alcohol breathalyzer) of alcohol use.

Specific Aim 2b: Examine agreement between measures of medication adherence (mobile technology-delivered video of medication taking, Medication Event Monitoring System, pill count, self-report).

Adolescence is a critical developmental stage involving marked elevation in alcohol initiation, progression to AUD, and development of significant, lasting adverse outcomes from use. Effective treatments must be developed for AUD in this especially vulnerable age range. The identification of a well-tolerated, effective pharmacological treatment would represent a significant advance and could yield tremendous public health impact. The proposed trial will provide critical data to evaluate NAC as a highly promising pharmacotherapy for adolescent AUD, and regardless of NAC versus placebo outcomes will provide key methodological guidance for future randomized controlled trials of pharmacotherapies for adolescent AUD.

2.0 Background

Adolescent Alcohol Use Disorder

Adolescence is a key period in the development of alcohol use disorder (AUD), with nearly 15% of youth meeting diagnostic criteria for AUD by age 18.[1] Heavy adolescent alcohol use is related to serious psychosocial problems, including comorbid psychopathology,[2,3] poorer academic success,[4] and detrimental neurocognitive consequences.[5-7] Furthermore, binge alcohol use patterns initiated during adolescence persist into adulthood and significantly increase risk for subsequent AUD and related problems.[8-10]

Limitations in Current Treatments

Decreasing alcohol use at this early stage could have significant long-term implications; however, efforts to prevent or decrease heavy alcohol use during adolescence have only been modestly effective,[11,12] with up to 86% of youth returning to use within 12 months following treatment.[13,14] While several medications have been approved by the Food and Drug Administration (FDA) as efficacious in treating adult AUD, minimal pharmacotherapy research has focused on adolescents, and there are no FDA approved medications for adolescent AUD.[15] This limits treatment options for this especially vulnerable age group, as safety and efficacy of medications for adolescents cannot be inferred from adult studies.[16] Evaluation of alternative and more efficacious treatments for adolescent AUD is warranted.

N-Acetylcysteine as a Candidate Pharmacotherapy

Safety, Accessibility, and Affordability

N-acetylcysteine (NAC), a prodrug of the amino acid cysteine, possesses a long-established safety record, with pediatric and adult FDA approval since 1963. NAC has been used safely for several decades in children and adults, often at doses greatly exceeding those proposed for our study. A meta-analysis of NAC studies found that this supplement was well tolerated, with generally mild, most commonly gastrointestinal, adverse effects that did not require treatment interruption.[17] Systemic allergic reactions to NAC have been observed, but only with intravenous administration.[18] Reflecting its safety profile, NAC is available over-the-counter as a supplement, which further increases its potential acceptability, accessibility, and affordability. Our team's extensive experience with NAC in substance using adolescents and adults, many of whom also drank alcohol frequently, has further supported its acceptability and tolerability in the proposed trial's target population.[19-26]

Mechanism in Substance Use Disorders

Glutamate has emerged as a therapeutic target in the treatment of addictions.[27] Repeated use of an addictive substance results in glutamate dysregulation in various brain regions, including the nucleus accumbens, a striatal structure critical to motivation and learning.[28,29] NAC administration restores glutamate homeostasis via upregulation of the glutamate GLT1 transporter, clearing excess glutamate from the accumbens and reducing substance seeking and self-administration.[30,31] A recent meta-analysis of seven clinical trials found that NAC was superior to placebo for craving reduction across substance use disorders,[32] and clinical findings from our group (see Preliminary Findings) indicate that NAC reduces use across a range of substances, including cannabis, alcohol, tobacco, and cocaine.[19-26] The strongest clinical

findings to date are adolescent-specific, with NAC more than doubling rates of abstinence compared to placebo in adolescents with cannabis use disorder (CUD).[21]

Alcohol-Specific Findings

A recent promising preclinical study found that alcohol-consuming rats who were administered NAC inhibited alcohol intake up to 70% compared to saline-treated rats ($p<.0001$).[33] The effect of treatment remained for 4 days post-treatment, showing that NAC administration generates a neurochemical effect extending well past its 1-hour half-life in rodents. A subsequent trial studying the effects of NAC on alcohol self-administration in rats showed an 81% decrease in alcohol consumption for the NAC-treated group compared to placebo, as well as reduced rates of reacquisition in rats that had been abstinent from alcohol for 17 days.[34] In a study of rats who underwent 30 days of chronic alcohol ingestion, NAC prevented an increase in pro-inflammatory cytokines and a decrease in anti-inflammatory cytokines in frontal and hippocampal regions.[35] In a study of zebrafish, NAC prevented the effects of acute alcohol exposure on behavioral and oxidative stress parameters.[36] To date, no human AUD clinical trials of NAC have been published; however, secondary analyses from our prior cannabis cessation trials suggest NAC effects may generalize to other substances including alcohol (see Preliminary Studies section).

Innovation

Directly Testing N-Acetylcysteine for Adolescent AUD

Alcohol is the most commonly used substance in adolescence. Despite the success of NAC increasing cannabis abstinence rates two-fold in adolescents with cannabis use disorder,[21] and recent preclinical findings suggesting NAC reduces alcohol use in rodents by up to 81%, there are no studies examining the effect of NAC on adolescent AUD. Effective interventions during adolescence could have substantial long-term implications by reducing acute and enduring negative social, academic, and cognitive consequences related to heavy teen drinking.[5] Earlier treatment during this vulnerable period could help prevent more severe, treatment-resistant AUD in adulthood.

Proof-of-Concept of Randomized Controlled Trial Methodology for Adolescent AUD

There is no currently established template for adolescent AUD pharmacotherapy efficacy trials. Only a handful of trials have been conducted in adolescents, with varying methodology and small sample sizes, leading to difficulty with interpretation. While adult trials may be informative, adolescents with AUD often differ from adults in alcohol use patterns, use-associated problems, and receptiveness to, as well as adherence with, treatment. Before proceeding to a Phase III efficacy trial in adolescents, a number of developmentally-informed methods must be embedded and evaluated for feasibility and value in a proof-of-concept trial. In addition to traditional, established, office-based clinical trial procedures, we propose utilizing mobile technology to improve data collection, engagement, and medication adherence. We additionally propose comparing the value of a cross-section of self-report and biological measures of alcohol use and related behaviors, to determine the most appropriate measures for inclusion as efficacy outcomes in Phase III trials.

Research Team

The multidisciplinary investigative team consists of experienced clinical researchers with complementary expertise in adolescent alcohol and other substance use disorders, mobile technology, developmental psychopathology, neuropsychological testing, and human laboratory paradigms, as well as extensive experience conducting randomized controlled trials evaluating pharmacological and behavioral interventions for alcohol and other substance use disorders. Some scientific contributions of direct relevance to the proposed work are summarized below.

Preliminary Studies

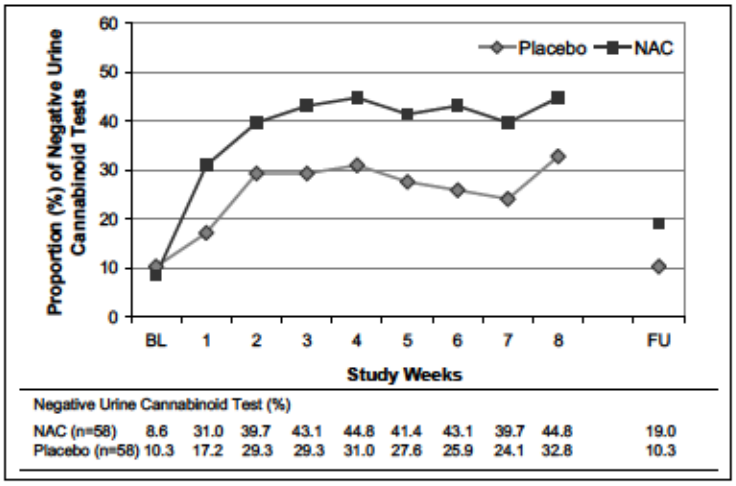
N-Acetylcysteine Treatment of Substance Use Disorders

Adolescent Cannabis Use Disorder

Our research team completed a double-blind randomized placebo-controlled trial of NAC for adolescent CUD.[21] Treatment-seeking youth with CUD (*N*=116) were randomized, in 1:1 parallel group allocation, to receive a double-blind 8-week course of NAC (1200 mg) or placebo twice daily, added to a contingency management intervention and brief weekly cessation counseling. A post-treatment follow-up visit occurred 4 weeks after treatment conclusion.

The primary study hypothesis was that participants randomized to NAC would have higher odds than those randomized to placebo to submit negative weekly urine cannabinoid tests (UCTs) during treatment. An intent-to-treat (ITT) approach including all randomized participants was used. In all analyses, participants lost to follow up or absent for visits were coded as having a positive UCT at every missed visit.

The proportion of negative UCTs in the NAC and placebo groups at each visit (ITT sample) is shown in the adjoining figure. The relationship between treatment and the odds of a negative UCT was OR=2.4 (95% CI: 1.1-5.4), p=0.021. Through the final treatment visit, 40.9% (190/464) of the UCTs among participants in the NAC group were negative, compared to 27.2% (126/464) among those in the placebo group. At the post-treatment follow-up visit (four weeks after medication discontinuation), 19.0% (11/58) of the UCTs among participants in the NAC group were negative, compared to 10.3% (6/58) among those in the placebo group. While still numerically favoring NAC, the overall treatment effect lost statistical significance at post-treatment follow-up (OR=2.2 [95% CI: 0.7-6.5], p=0.155), though the trial was not powered for this outcome.



Negative Urine Cannabinoid Test (%)										
NAC (n=58)	8.6	31.0	39.7	43.1	44.8	41.4	43.1	39.7	44.8	19.0
Placebo (n=58)	10.3	17.2	29.3	29.3	31.0	27.6	25.9	24.1	32.8	10.3

A *post hoc* sensitivity analysis was performed on odds of negative UCTs during treatment, using multiple methods to manage missing data and participant dropouts. The selection of missing data handling had little effect on outcomes.

Every efficacy outcome approach explored, inclusive of both UCT and self-report data sets, consistently yielded findings numerically favoring NAC over placebo. Statistically significant differences were noted in the study's *a priori* outcome analysis (odds of negative weekly UCT over the course of treatment) and in the end-of-treatment binary UCT outcomes (last 2 weeks [OR 2.55, p=0.036] and last 4 weeks [OR 2.59, p=0.045]). Odds ratios were greater than 2 for all end-of-treatment (last 2 and last 4 weeks) UCT and self-report outcomes. Furthermore, there was strong agreement between UCT and self-report. Overall, findings demonstrated consistently favorable outcomes for NAC versus placebo across a wide variety of approaches.

Alcohol Use Disorder

To date, no published clinical trials have examined the effect of NAC on AUD. Therefore, secondary analyses were performed on the adolescent cannabis cessation dataset described above to examine the effect of NAC on co-occurring alcohol use over the 8-week treatment course.[25] Less cannabis use (based on urine cannabinoid tests and creatinine adjusted cannabinoid levels) was associated with less alcohol use in the NAC treated group but not in the placebo treated group (interaction t_{86}=2.44, p=0.016). In sum, participants who reduced cannabis use while using NAC also had a significant reduction in alcohol use; this was not the case in the placebo condition, supporting the assertion that NAC may be exerting effects across substances, including alcohol. Findings are notable considering this sample was not attempting to reduce alcohol use and was not receiving a combined behavioral treatment for alcohol use.

We recently completed a 12-week, multi-site cannabis cessation trial examining the effects of NAC versus placebo on adults with CUD.[22] Participants (*N*=302, ages 18-50) were randomized to double-blind NAC (1200mg, twice daily) or placebo. Neither alcohol use nor desire for alcohol cessation were requirements for participation. Participants that returned for at least one treatment visit and had recorded alcohol use data (*n*=277) were included in secondary analysis of alcohol use.[26] Compared to the placebo group, participants in the NAC group had increased odds of between visit alcohol abstinence [OR=1.37; 95% CI=1.06-1.78; p=0.019], fewer drinks per week [RR=0.67; 95% CI=0.48-0.99; p=0.045], and fewer drinking days per week [RR=0.69; 95% CI=0.51-0.92; p=0.014]. Changes in concurrent cannabis use amounts were not correlated to any of the alcohol use variables. These findings indicate that, even without alcohol treatment-seeking or psychosocial interventions targeting alcohol use, NAC may be effective at reducing consumption of alcohol by ~30% among individuals seeking treatment for CUD.

A pilot clinical trial at MUSC examined the effects of NAC for reduction of alcohol and drug craving and posttraumatic stress among Veterans (*N*=35) with comorbid substance use disorder and trauma.[19] Though an AUD diagnosis was not required for inclusion, 82% of the sample met criteria for an AUD. NAC yielded average reductions in the amount and frequency of alcohol and drug craving that were more than 2.5 times the magnitude of placebo effects.

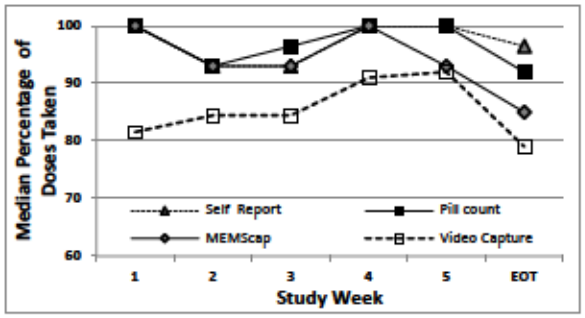
In another double-blind, placebo-controlled trial of NAC in adults with cocaine use disorder (N=111), NAC was associated with significantly longer time to relapse and lower craving as compared to placebo among participants entering the trial abstinent.[23] Secondary analysis of individuals in the study who met criteria for AUD (*n*=28/111) revealed that the NAC treated group consumed approximately 9 fewer drinks per week (i.e., 46% less) than the placebo treated group (NAC = 10.4 standard drinks per week vs. placebo = 19.3 standard drinks per week) (Group × Time Wald Chi-Square = 15.9, *df*=8, $p<0.05$). This work provides further support for the therapeutic potential of NAC in reducing alcohol use.

Bolstered by the aforementioned preliminary findings, our team is examining NAC more directly for AUD, in a within-subjects crossover mechanistic/neuroimaging study of non-treatment-seeking adolescent heavy drinkers (K23AA025399) and in another trial in adults with co-occurring AUD and posttraumatic stress disorder (R01AA025086). The proposed trial does not duplicate or overlap with the methods of these two studies, and instead will serve to complement them to better inform future work, including potential Phase III trials with NAC in AUD. Conducting this work in parallel, rather than in series, will yield synergy to more efficiently proceed with important next steps in treatment development.

In sum, our work with NAC has demonstrated significant treatment effects for CUD in adolescents, and secondary examination across multiple trials of NAC for substance use disorders has revealed reductions in alcohol use over the course of treatment, reinforcing promising alcohol-specific preclinical findings (see *Alcohol-Specific Findings*). Combined, these results indicate that a proof-of-concept clinical trial of NAC for adolescent AUD is warranted.

Incorporation of Mobile Technology to Enhance Clinical Trials

Alcohol use is often underestimated over longer periods of recall.[37,38] Our team has experience utilizing mobile surveys as a means of daily data collection that integrates with an existing database management and data capture system, Research Electronic Data Capture (REDCap).[39] In several ongoing protocols (R01DA042114, R34DA042228, UG3DA043231; K23AA025399) we obtain daily morning reports of past-day substance use via REDCap.[40] Participants receive a survey link via text message and email once per morning during the study protocol asking them to report their alcohol and other substance use over the past 24 hours to minimize bias due to retrospective recall.



Additionally, medication adherence is significantly over-estimated when relying on participant self-

report.[41,42] Medication non-adherence is extremely detrimental to the success of pharmacological clinical trials, as poor adherence (particularly when overestimated) may lead investigators to incorrectly assume that a potentially useful medication is ineffective. Also using REDCap, we have successfully implemented video capture to observe medication-taking in our clinical trials (R01DA042114, R34DA042228, UG3DA043231, K23AA025399). See figure for week-by-week comparison of median percentage of doses reported as taken among adherence assessments from UG3DA043231 (a clinical trial of a twice-a-day medication for CUD in adults), indicating that traditional measures may overestimate adherence, compared to the objective video capture method. For the video capture procedure, participants receive a link twice daily to their mobile phone. They record themselves taking the medication in real-time and the video is uploaded for later staff verification. Completion rates are over 70% in one study[40] and considering that the medication video is likely a conservative estimate of medication adherence (i.e., participants may take the medication without recording a video), we believe this represents a significant improvement over other clinical trials in which biomarkers suggest study-defined medication non-adherence may sometimes be as high as 40-55%.[41,43] The same procedures will thus be utilized in the proposed trial to confirm and encourage medication adherence.

Summary

The work outlined above demonstrates the expertise of the investigative team in a number of areas directly relevant to the proposed project. The results of the preliminary studies are encouraging and indicate that further investigation of NAC in adolescents with AUD is warranted. Building on the previous NAC studies by our group, we propose to conduct a clinical trial focused specifically on adolescents with AUD. This experienced team brings together a unique set of skills and a strong track record of productivity to ensure the success of the proposed project.

3.0 Intervention to be studied

N-acetylcysteine (NAC), a prodrug of the amino acid cysteine, possesses a long-established safety record, with pediatric and adult FDA approval since 1963. NAC has been used safely for several decades in children and adults, often at doses greatly exceeding those proposed for our study. A meta-analysis of NAC studies found that this supplement was well tolerated, with generally mild, most commonly gastrointestinal, adverse effects that did not require treatment interruption.[17] Systemic allergic reactions to NAC have been observed, but only with intravenous administration.[18] Reflecting its safety profile, NAC is available over-the-counter as a supplement, which further increases its potential acceptability, accessibility, and affordability. Our team's extensive experience with NAC in substance using adolescents and adults, many of whom also drank alcohol frequently, has further supported its acceptability and tolerability in the proposed trial's target population.[19-26]

Glutamate has emerged as a therapeutic target in the treatment of addictions.[27] Repeated use of an addictive substance results in glutamate dysregulation in various brain regions, including the nucleus accumbens, a striatal structure critical to motivation and learning.[28,29] NAC administration restores glutamate homeostasis via upregulation of the glutamate GLT1 transporter, clearing excess glutamate from the accumbens and reducing substance seeking and self-administration.[30,31] A recent meta-analysis of seven clinical trials found that NAC was superior to placebo for craving reduction across substance use disorders,[32] and clinical findings from our group (see Preliminary Findings) indicate that NAC reduces use across a range of substances, including cannabis, alcohol, tobacco, and cocaine.[19-26] The strongest clinical findings to date are adolescent-specific, with NAC more than doubling rates of abstinence compared to placebo in adolescents with cannabis use disorder (CUD).[21]

Consistent with our established procedures, United States Pharmacopeia (USP) grade NAC powder will be encapsulated in 600 mg quantities (two 600 mg capsules per dose). Matched placebo capsules will also be prepared. Randomized participant will be instructed to take 1200 mg twice daily, in approximately twelve-hour intervals. This dose was chosen due to its demonstrated tolerability and evidence of effect on

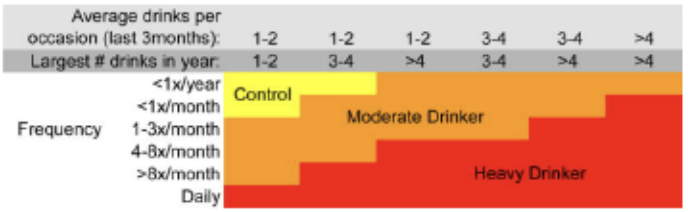
substance use in our prior trials. Participants will be expected to continue taking study medication until they come in for the Week 8 (End-of-Treatment) Visit.

4.0 Study Endpoints

The primary efficacy endpoint is reduction in alcohol use (total standard drinks), compared between NAC and placebo groups. Additional outcomes of interest include agreement between self-report (mobile technology-delivered daily self-report, Timeline Follow-Back) and biomarkers (ethyl glucuronide, carbohydrate deficient transferrin, phosphatidylethanol, alcohol breathalyzer) of alcohol use, as well as agreement between measures of medication adherence (mobile technology-delivered video of medication taking, Medication Event Monitoring System, pill count, self-report).

5.0 Inclusion and Exclusion Criteria/ Study Population

Inclusion Criteria



Participants must be age 13 to 25, must be able to understand the study and provide written informed consent (for those under age 18, parent/legal guardian must be able to provide consent and participant must be able to provide assent), must be a current moderate or heavy drinker by established adolescent criteria (see figure),[49] must meet criteria for current AUD and express interest in AUD treatment, and (individuals assigned female at birth) must agree to use appropriate birth control methods during study participation: oral contraceptives, contraceptive patch, barrier (diaphragm or condom), levonorgestrel implant, medroxyprogesterone acetate, complete abstinence from sexual intercourse, or hormonal contraceptive vaginal ring. Participants must also have successfully completed the YC Intake Protocol (PRO#94743) within approximately 30 days of formal study screening.

Exclusion Criteria

Participants must not score >10 on the Clinical Institute Withdrawal Assessment for Alcohol,[50] as this would warrant more acute medical intervention than offered in the trial. Participants must not have known allergy or intolerance to NAC, no current use of *N*-acetylcysteine or any supplement containing *N*-acetylcysteine (must agree not to take any such supplement throughout study participation), individuals who are pregnant, lactating, or contemplating pregnancy/lactating over the next 6 months, must not be currently enrolled in ongoing treatment for AUD, and any other medical or psychiatric condition or other significant concern that in the Investigator's opinion would impact participant safety or compliance with study instructions, or potentially confound the interpretation of findings.

Co-Occurring Disorders

Consistent with our established inclusion/exclusion protocols across adolescent substance use disorder treatment trials, we elected not to exclude non-acute co-occurring mental or substance use disorders, as these are so common among adolescents with AUD.[51] Inclusion will allow for more potential generalizability of the study's findings, and our established policies and procedures have demonstrated the team's ability to safely manage adolescent participants with co-occurring disorders. We will monitor mental health and substance use measures throughout the trial, and participants will meet with the medical clinician for safety assessments at all visits. We will additionally provide consistent availability and involvement of the MPIs and clinician Co-Is (all with adolescent mental health and substance use expertise).

Age Range

The participant age range for the proposed study (13-25 years old) has broad agreement and support, and was chosen for multiple reasons. The Maternal Child Health Bureau defines adolescence as age 11-21, and the Center for Disease Control and Prevention defines it as ages 10-24. It is increasingly understood that adolescent brain development continues through age 25.[69] While some youth initiate alcohol use before

age 13, few exhibit sufficient symptoms of AUD to merit study inclusion, so we concluded that a range of 13-25 would be appropriate.

We recognize that the developmental context varies considerably even within the narrowed range of 13-25. To address this, we plan to stratify randomization by secondary school status, ensuring that equivalent proportions of those in secondary school (e.g., middle school, high school) and those not in secondary school (e.g., dropped out, graduated, working, attending college) are represented in the treatment randomization groups. We deemed the psychosocial developmental differences between these groups as more meaningful than those between arbitrary ages within the inclusion range to serve as a point of stratification. Given our prior success, we anticipate recruiting similar proportions of youth across these strata, and we will adjust strategies if the proportions become skewed. This stratified approach will convey the added opportunity to compare outcomes (in exploratory fashion) between these developmental subgroups.

Inclusion of Women and Minorities

1. **Targeted/planned distribution of participants by gender and racial/ethnic groups.** The enrollment demography was aimed to match the demographic composition of Charleston County. Of the total 120 participants to be recruited and followed in this study, 50% are female; 10% are Latino/a ethnicity.
2. **Subject selection criteria and rationale for selection of sex/gender and racial/ethnic group members**. Adolescents will be recruited based on previously successful techniques used for several large-scale adolescent substance use disorder clinical trials conducted by the research team, including a mix of community and multimedia advertising as well as clinical recruitment. The gender, ethnic, and racial composition of the sample will be based on the demography of Charleston County. Racial composition is: 67% White, 29% African American, 2% multiracial, 1% Asian, <1% American Indian/Alaskan Native, and <1% Native Hawaiian or Other Pacific Islander.
3. **No gender or racial/ethnic group will be excluded.**
4. **Outreach programs for recruiting sex/gender and racial/ethnic group members**: To recruit representative numbers in each demographic category, we can focus recruitment efforts at communities that have higher proportions of any underrepresented demographic group.

Inclusion of Children

1. **Rationale for the specific age ranges of children to be included.** All participants in this study will range in age from 13 to 25 and will therefore include a substantial portion of children (NIH definition of <18 years old). Evaluation of alternative and more efficacious treatments for adolescent substance users is warranted. N-acetylcysteine (NAC) is an over-the-counter supplement with a strong safety and tolerability profile for substance-using adolescents. This study is a step in determining if NAC could be a potential treatment option for adolescents with alcohol use disorder.
2. **Expertise of study team for conducting research with children.** PI Dr. Kevin Gray is a board-certified psychiatrist and nationally recognized expert in the area of adolescent substance use disorders and treatment. Dr. Gray will be responsible for responding to any mental health or medical referrals that may arise.
3. **Facilities available to accommodate children.** The investigative team has a strong record of adolescent substance use research using the described techniques, including clinical trials using N-acetylcysteine. Studies are performed in an environment that is appropriate and safe for youth. The office suite is a locked facility with 24-hour security monitoring. Private testing areas, including a physical exam room, are available for study use. There is a separate waiting area to accommodate families.
4. **Sufficient number of children to contribute to a meaningful analysis.** 120 participants will be enrolled in this 8-week, intent-to-treat, double-blind, randomized placebo-controlled proof-of-

concept trial of N-acetylcysteine. This sample size is sufficient to achieve the statistical power necessary to the meet the study aims.

6.0 Number of Subjects

We plan for 120 total randomized participants in the study (excluding screen failures and individuals dropping out prior to randomization).

7.0 Setting

The study will be conducted in our outpatient research clinic at MUSC or remote/ hybrid via doxy.me, WebEx, or Zoom.

8.0 Recruitment Methods

Recruitment Strategies

All participants will be recruited from a pool of participants who have completed an ongoing Entryway Intake study (PRO #94743). Participants who have completed this protocol and who appear to meet eligibility criteria will be offered participation in this protocol. Individuals who participate in the Entryway Intake consent for their data to be carried forward into the study in which they ultimately enroll. The Entryway Intake includes, but is not limited to, a comprehensive substance use history assessment and a structured diagnostic interview for psychiatric conditions, including assessment of AUD and other substance use disorders, as well as bioassay collection. After completion of the Entryway Intake, participants who are eligible for the current study will be offered participation. They will consent to sharing data collected across both protocols for analysis. Examples of shared data include, but are not limited to vitals, study enrollment and study completion status. Since launching the shared Entryway Intake in September 2020, we have averaged 33 referrals per month of youth (ages 16-22) interested in participating in alcohol studies. Our team is highly experienced in recruiting and retaining at-risk and substance-using youth. Adolescents (N=120) will be recruited from the community, schools, and clinical settings. Though this population is notoriously difficult to engage, our clinical trials (5 completed pharmacotherapy trials to date targeting adolescent substance use disorders, with collective N=460) have consistently enrolled and retained participants well ahead of goal rates (generally $\geq$4 adolescents per month). We have an aggressive plan for recruitment and are confident that our previously established strategies will ensure success. Assuming an initial 3-month period for study setup, per-participant study duration of 6 months, and a concluding 3-month period for data analysis and publication, we anticipate a recruitment window of 48 months. To meet the recruitment goal, we must enroll 6 participants per month, which is feasible, realistic, and consistent with our past efforts. Study staff may also use the following methods of advertising to promote recruitment: local publications, physician offices/local clinics, MUSC campus, local college and high school campuses as permissions are granted by the academic institution, internet, social media, TV commercials, and other locations (e.g., restaurants, movie theaters, malls, buses/transportation services) in the community that agree to post Brand and IRB approved recruitment materials for this study and that may reach our target population.

Our confidence in successful recruitment stems from a complement of strategies developed over the past 15 years of experience with randomized controlled trials of pharmacotherapies and behavioral treatments for adolescents with substance use disorders. We have had consistent success via multimedia and internet/social media advertisements, referrals from clinics, and respondent-driven sampling/participant peer referral. Across large-scale studies of adolescents with tobacco (R01DA17460, U01DA031779), cannabis (R01DA026777, R01DA042114), and alcohol (K23AA025399) use disorders, we have consistently met or exceeded the goal enrollment rate planned for the proposed trial. For the proposed study, we plan to continue our established combined approach, which we are confident will be successful. Additionally, a Youth Collaborative of adolescent substance use studies within the Division has been developed that will not only

allow for the cost sharing needs of advertising and study promotion but also make the process of identifying opportunities at MUSC easier for participants, parents/family, and community partners. By marketing a central point of contact for interested parties to seek additional information, callers can be triaged to the appropriate study or directed to clinical services based on their expressed interest level and/or prescreen outcomes. This is particularly appropriate for incorporation since there is a high likelihood of polysubstance use. By streamlining the referral process, it lessens the burden on the caller to duplicate their efforts and potentially become fatigued and disillusioned with the process and ultimately not receive the service delivery that they desire.

9.0 Consent Process

Pre-Screening Assessment

Individuals responding to recruitment materials or otherwise referred to the study will be pre-screened on the phone or in person to ascertain preliminary eligibility status. A series of questions will determine preliminary eligibility, and formal screening appointments will be scheduled for those who meet these eligibility criteria. No information obtained during the pre-screening will be used as research data.

Informed Consent Procedures

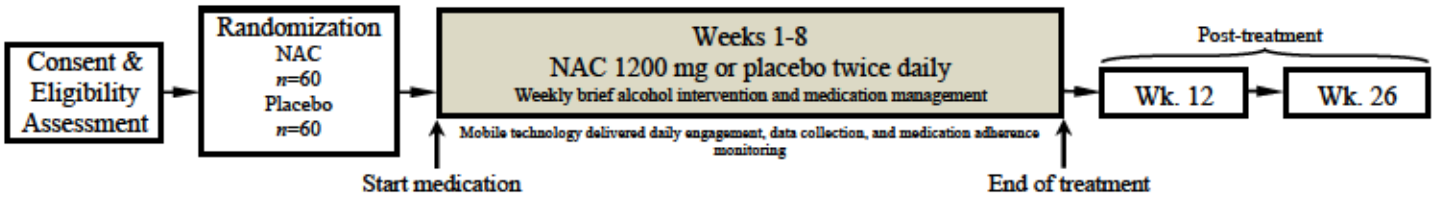
Prior to the initiation of any study procedures, written informed consent and HIPAA authorization will be obtained by the designated research staff. Informed consent/assent may be obtained either in person in the research office, or remotely via REDCap and doxy.me, WebEx, or Zoom. Parents/guardians of participants under 18 years old will participate with the adolescent in the screening, evaluation, and informed consent/assent procedure. Participants 18 years and older will be able to provide their own informed consent. The informed consent process will include a thorough discussion of potential risks associated with participation, including potential adverse effects of study medication. The complex issues of informed consent and assent, and related limitations of confidentiality, as they apply to adolescents and their parents/guardians, are understood by the investigative team and will be communicated clearly during the telephone screening and assessment visit. In the case of adolescents in South Carolina Department of Social Services custody, state guidelines regarding consent for clinical research participation will be followed.

Potential participants (and parents/guardians, as appropriate) will be given a copy of the IRB-approved consent form and asked to read it either on site or at home in accordance with the consent process. Those who demonstrate understanding of the study and voluntarily agree to participate will be asked to sign the informed consent form and proceed with the screening assessments.

10.0 Study Design / Methods

Overview

The proposed study is a Phase II, 8-week, double-blind, randomized, placebo-controlled, proof-of-concept trial of NAC for AUD in adolescents (N=120). Outcome measures will allow assessment of NAC effects on alcohol use, while the study will additionally evaluate the feasibility and utility of implementing developmentally-informed methods for adolescent AUD pharmacotherapy trials.



Screening/Baseline Assessment

After consenting to participate in the study, participants will start the screening/baseline assessment phase. Ideally, the screening/baseline assessment procedures will be completed in one in-person or remote

visit, but they can be completed in more visits if necessary. Participants will provide general demographic information and complete a locator form to optimize between-visit contact and communication. The comprehensive assessment will include a medical history and physical examination, urine drug testing, semi-structured psychiatric diagnostic interview, and detailed assessment of alcohol and other substance use history. Should a participant have screen failed for protocol #54499, N-Acetylcysteine for Youth Cannabis Use Disorder, the data/information that was completed as part of that protocol could be incorporated into this protocol if the participant presented as a likely eligible candidate. The participant would need to confirm interest and consent to such participation and data sharing. Data/information from PRO#94743 is also transferable as it serves as the point of intake into the Youth Collaborative, of which this protocol is a member study. The screening procedures of these protocols closely mirror each other, and this data sharing would remove the need for duplication of procedures as well as undue burden and testing fatigue.

Randomization

Following completion of screening/baseline assessments and determination of study eligibility, participants who return for the randomization visit and continue to be eligible will be randomly assigned to one of the two conditions (NAC or placebo) for 8 weeks of treatment. Random assignment will be on a 1:1 ratio to one of the two conditions. Randomization will be stratified by school status [in secondary school (i.e., middle or high school) versus not] and by sex (female versus male). Adolescents no longer in secondary school (i.e., dropped out, graduated, working, and/or enrolled in college) may have different psychosocial contexts and contingencies related to alcohol use, compared to those still in secondary school. The sex stratification will help avoid significantly discrepant representation of sex across treatment groups, allowing for exploration of potential sex differences in study outcomes. We considered additional stratification variables (e.g., severity of AUD, co-occurring other substance use disorders, history of attention-deficit/hyperactivity disorder), but, amid concern over excessive division of participants among multiple cells, judged that these could be adequately explored as covariates during efficacy analyses.

Mr. Baker, the study statistician, will generate the randomization schedule using a stratified permuted random block design with block sizes of 2, 4 and 6 to ensure lack of predictability along with relative equality of assignment across treatment groups. All study staff, the investigative team, and participants will remain blind to study assignments until the completion of the study, unless an unblinding is deemed medically necessary. Mr. Baker will review randomization data on a regular basis to ensure that the scheme is being implemented according to plan. A randomization slot, once used, will not be re-allocated.

Treatment

Psychosocial Intervention

All participants will receive 8 weekly brief alcohol intervention sessions from NIAAA's brief alcohol intervention,[52] which includes motivational interviewing and setting individual goals and action plans, consistent with the current standard-of-care treatment.

Pharmacological Intervention

Consistent with our established procedures, United States Pharmacopeia (USP) grade NAC powder will be encapsulated in 600 mg quantities (two 600 mg capsules per dose). Matched placebo capsules will also be prepared. If assessment procedures reveal that a participant meets inclusion criteria and none of the exclusion criteria, the participant will be randomized to double-blind NAC or matched placebo. The participant will be given a supply of medication, with instruction to take 1200 mg twice daily, in approximately twelve-hour intervals. Remote participants will receive medication supply in the mail. This dose was chosen due to its demonstrated tolerability and evidence of effect on substance use in our prior trials. Subsequent medication supply will be given out or mailed a week in advance to decrease the risk of the participant running out of study medication. Participants will be expected to continue taking study medication until they their Week 8 (End-of-Treatment) Visit.

Multiple methods for medication adherence assessment may be implemented, including participant self-report via mobile daily diaries, weekly pill counts, remote adherence monitoring system (e.g. Pillsy®), and mobile technology-delivered video capture of medication taking (see Preliminary Studies).40 Pillsy caps fit securely on medication bottles and provide a time-stamp each time the bottle is opened. The Pillsy cap also displays the number of times the bottle has been opened within a 24-hour period to assist participants in remembering to take both daily doses. Participants will be reminded to take medication and upload medication-taking videos via twice-daily mobile technology-delivered text messages, generated using the REDCap system and timed based on the participant's typical daily schedule.40 If participants do not have a compatible mobile phone, one will be provided for use in completing mobile technology-facilitated procedures.

Medication tolerability and effects will be systematically assessed. Participants will be encouraged to contact study personnel between visits to address any immediate concerns regarding adverse effects. If a participant experiences intolerable medication-related adverse effects at any point during the study, a dose reduction to 600 mg twice daily may be undertaken. The dosage may be increased back to 1200 mg twice daily at the discretion of the medical clinician. However, if a participant is unable to tolerate the reduced dose, medication will be discontinued, and the participant will continue to come in for study visits.

Post-Treatment Follow-Up

Post-treatment follow-up visits will be conducted at approximately 12 weeks and 26 weeks from initial randomization.

Retention Strategies

Retention, like recruitment, is a considerable challenge in clinical trials for adolescent substance use disorders. Given intent-to-treat analysis (including all randomized participants and assuming that participants are using alcohol at each missed visit), poor retention may result in an underestimate of treatment effects and the need to impute missing data. It also limits participant exposure to treatment, which further attenuates treatment effects.

Our team has been successful with optimizing participant retention via a number of well-established strategies. Our research clinic is centrally located and open for extended (including after-school) hours, with free parking immediately in front of the building. All of our visits can also be completed remotely via doxy.me, WebEx, or Zoom. We maintain active communication with participants between visits via their preferred mode of contact (e.g., text message, telephone, e-mail). We also strictly maintain confidentiality, a particularly significant issue in studies focused on interventions for alcohol and other substance use in youth. Dr. Gray (PI), a board-certified child, adolescent, and general psychiatrist, is well versed in managing communication with adolescents and parents/guardians while maintaining appropriate bounds of confidentiality and managing issues of safety; the resultant trust and rapport supports participant adherence and retention. Additionally, we strive to reinforce participants appropriately for attending visits and completing study procedures. Higher magnitude reinforcement is associated with significantly improved retention,[44-46] and cash rewards (relative to gift cards or other tokens) are associated with improved session attendance.[47] Adult and adolescent studies reveal that high-magnitude rewards are generally not used to purchase substances of abuse.[47,48] In the proposed trial, participants will be compensated $20 for each standard visit and $40 for visits that involve cognitive testing. Participants will also be eligible for as much as $20 per week during the 8 weeks of active treatment for completion of remote data entry via smartphone, including video uploads confirming twice-daily medication adherence. A bonus of $60 is available for completing the Week 8 end-of-treatment visit and completing study requirements of phone use. If the participant is enrolled directly from the Youth Collaborative Intake (PRO 94743) with data from that protocol being utilized for the current aims, this study may be responsible for the $40 Intake Screening

compensation. Participants will be eligible for a maximum of $600 for uninterrupted completion of all study visits and procedures.

Remote Visits – The informed consent/assent procedure can be conducted remotely via REDCap and Zoom. Participants will complete direct-entry REDCap surveys remotely. The study medical clinician will have the option to conduct assessments and cessation counseling remotely via Zoom or phone. All visits taking place can be conducted entirely remotely via REDCap, Zoom, or phone. Participants may receive study medication by mail. The participant will also be provided supplies required to complete an offsite/at home urine collection and CLIA waived qualitative EtG test, UDS, and pregnancy test for participants assigned female at birth, the results of which will be verified using video and photo capture. The alcohol breathalyzer test and the blood draw will not occur during remote visits.

In addition, a hybrid model can be utilized if an in-person participant has an unexpected conflict with attending an in-person visit (e.g., transportation issue or travel), arrangements may be made to remotely complete visit procedures, in order to maintain data collection and study engagement. In very rare instances, study medication may be shipped to a participant when extended or indefinite relocation from the study center would prevent regularly planned medication management (dispensing/accountability and compliance) at an in-clinic visit. For remote or hybrid participants medications will be shipped in proper packing with tracking to protect medication as well as participant privacy. Prior to the mailing of any materials, ongoing participant interest in continued study participation will be confirmed, dosing instructions and safety needs will be reviewed, and depending on the ability to physically return study medication/blister packs, appropriate destruction measures will be provided. If such remote measures are employed, participants will be directed to have more extended visualization of their medication blister packs in their video recording of study medication dosing to allow study personnel to properly monitor medication accountability.

In our prior NAC trial for CUD, with the above-mentioned strategies in place, among participants presenting for any visits after medication initiation, 84% completed the entire course of treatment. We maintain similar success in our ongoing studies focused on adolescent tobacco (U01DA031779), cannabis (R01DA042114), and alcohol (K23AA025399) use disorders.

Instruments/Measures/Materials (see **Study Timetable** for an overall summary)

Clinical Assessments

The instruments to be used were selected because many are standardized, have good psychometric properties, are widely used, and have been used by our research team.

Timeline Follow-Back, inclusive of detailed quantification of alcohol use, measured in standard drinks, will be conducted at baseline to characterize self-reported alcohol and other substance use.[53] This approach will be enhanced during trial participation by the implementation of mobile technology-delivered daily alcohol and other substance use reports, using methods established by our research team (see Preliminary Studies).[40] At baseline assessment and during each daily survey, participants are shown visuals and provided the definition of a standard drink to aid in reporting. The survey expires at midnight so that participants cannot access an old survey. A traditional Timeline Follow-Back will be conducted during laboratory visits to fill in missing data. These alcohol use quantification strategies will be used in calculating primary efficacy outcome measures, including total standard drinks, compared between the 4 weeks prior to intake and the last 4 weeks of study treatment. The trial is powered to test this primary outcome (see *Statistical Methods*), and other alcohol use-related outcomes are exploratory at this Phase II stage of investigation.

The MINI International Neuropsychiatric Interview will be conducted at the initial assessment visit to evaluate for psychiatric and substance use disorders.[54,55] Throughout the study, a variety of self-report and clinician-administered measures (summarized in the **Study Timetable**) will be collected. In general, these measures will be used to assess and track mental health, substance use-related problems, quality of life, and other functional outcomes over the course of study participation as exploratory aims. Adverse events will

be documented, rated for severity and relatedness, coded in Medical Dictionary for Regulatory Activities (MedDRA) terms, and managed/reported appropriately via established procedures (see Human Subjects section). To evaluate penetration of the blind, participants and the primary study personnel who interact with the participant will be asked at multiple time points whether they think the participant is receiving NAC or placebo.

Laboratory Tests

Combined use of ethanol metabolites and traditional biomarkers [i.e. alcohol breathalyzer, urine ethyl glucuronide (EtG), blood carbohydrate deficient transferrin (CDT), and blood phosphatidylethanol (PEth)] will be utilized to monitor drinking and corroborate self-report measures.[56-59] Urine drug tests (comprehensive panel testing for EtG, cocaine, cotinine, THC, K2, amphetamines, opioids, and benzodiazepines) will be used to monitor other substance use. Urine pregnancy tests will be conducted with participants assigned female at birth. If available when the trial is conducted, we will incorporate wearable alcohol biosensors (e.g., BACtrack Skyn™) as another potential biomarker of use, though at this stage basic questions of participant acceptability (willingness to wear) and data management/interpretation (best methods to assess and compare with self-report and other biomarkers) are not yet addressed; this would be exploratory, though potentially highly valuable in a proof-of-concept trial.

Cognitive Task Performance Assessments

CNS Vital Signs, a computer-administered battery of performance tests, assessing subtle changes in mental acuity, learning and memory, psychomotor speed, complex attention, impulsivity, planning and sequencing, and other aspects of performance, will be administered at baseline, during treatment, at treatment conclusion, and at post-treatment follow-up.[60] A training/practice session will be conducted during screening. The performance battery, validated among adolescents, requires approximately 45 minutes to complete and includes the following components: *Verbal Memory Test (Part 1), Visual Memory Test (Part 1), Finger Tapping Test, Symbol Digit Coding, Stroop Test, Shifting Attention Test, Continuous Performance Test, Perception of Emotions Test, Reasoning Test, Four Part Continuous Performance Test, Dual Task Test, Verbal Memory Test (Part 2), Visual Memory Test (Part 2)*. We have utilized this battery in our prior work with NAC in adolescents with CUD, demonstrating evidence of performance changes in some cognitive domains with abstinence versus continued use.[61]

Study Timetable

	SC	Double-Blind Medication Phase(± 3 day visit window)									FU(±5 day visit window)	
Week →	SC	0[@]	1	2	3	4[&]	5	6	7	8[#]	12	26/ET
Informed Consent	X											
Locator Form and Updates	X[!x]	X	X	X	X	X	X	X	X	X	X	
Demographics w/MacArthur Social Ladder	X[!x]											
Technology Survey			X									
Beliefs about Medicines &Medication Adherence Video Feedback Form										X		X>
Medical Assessments												
History and Physical	X[%!]											
Height, Weight, Blood Pressure, and Pulse	X[%!]					X				X		X
Clinical Institute Withdrawal Assessment of Alcohol-Revised (CIWA)	X[%]	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X[%]	X	X	X	X	X	X	X	X	X	X	X
Prior/Concomitant Meds	X[%!]	X[$]	X	X	X	X	X	X	X	X	X	X
Penetration of Blind Assessment						X				X		X
Clinical Global Impression of Severity Scale (CGI-S)	X[%]					X				X		X
Alcohol Use Disorder Outcomes	X[%]					X				X		X
Psychological Assessments												
MINI International Neuropsychiatric Interview	X[%!x]											
Barratt Impulsiveness Scale	X[!]											
Short UPPS-Y Impulsivity Scale	X[!x]											
Current Quality of Life	X[!]					X				X		X
ShortForm36 Health Survey (SF36)	X					X				X		X
Modified Risk Behavior Survey	X					X				X		X
Beck Depression Inventory	X[!]	X				X				X		X
Beck Anxiety Inventory	X[!]	X				X				X		X
ADHD Rating Scale		X				X				X		X
Pittsburgh Sleep Quality Index	X[!x]					X				X		X
Columbia Suicide Severity Rating Scale	X[!]	X[$]				X				X		X
Cognitive Task Performance Testing	X[#]	X				X				X		X
Treatment Services Review	X	X	X	X	X	X	X	X	X	X	X	X
Substance Self-Report												
Alcohol and Other Substance Use History	X[!x]											
SOCRATES	X[x]	X				X				X		X
Modified Monitoring the Future Questionnaire	X											
Drinking Motives Questionnaire	X											
Rutgers Alcohol Problem Index	X[x]					X				X		X
Alcohol and Other Substance Use Daily Reports, Confirmed with TLFB	X[%!x]	X[$]	X	X	X	X	X	X	X	X	X	X
Obsessive Compulsive Drinking Scale	X	X	X	X	X	X	X	X	X	X	X	X
Alcohol Purchase Task	X	X				X				X	X	X
Marijuana Adolescent Problem Index, *cannabis users only*	X[!x]					X				X		X
Modified Fagerström Tolerance Questionnaire (mFTQ), *tobacco users only*	X[!x]					X				X		X
Penn State E-cig Dependence Index, *e-cig users only*	X[!x]					X				X		X
Lab Samples/Testing												
Alcohol Breathalyzer	X[x]	X	X	X	X	X	X	X	X	X	X	X
Urine Ethyl Glucuronide (EtG)	X	X	X[⊕]	X[⊕]	X[⊕]	X[⊕]	X[⊕]	X[⊕]	X[⊕]	X[⊕]	X[⊕]	X[⊕]
Blood Carbohydrate Deficient Transferrin (%CDT)	X					X				X		X
Blood Phosphatidylethanol (PEth)	X					X				X		X
Urine Drug Test	X[!x]	X	X[⊕]	X[⊕]	X[⊕]	X[⊕]	X[⊕]	X[⊕]	X[⊕]	X[⊕]	X[⊕]	X[⊕]
Urine Pregnancy Test[‡]	X[!x]	X[$]	X*[⊕]	X*[⊕]	X*[⊕]	X[⊕]	X*[⊕]	X*[⊕]	X*[⊕]	X[⊕]		
Psychosocial Procedures												
Medication Adherence Assessment (Clinic Visit Self-Report, Pill Count, Pillsy Cap, Daily Reports, and REDCap Video Review)			X	X	X	X	X	X	X	X		
Brief Alcohol Intervention		X	X	X	X	X	X	X	X			
Medication Management		X	X	X	X	X	X	X	X			

	SC	Double-Blind Medication Phase(± 3 day visit window)									FU(±5 day visit window)	
Week →	SC	0ø	1	2	3	4&	5	6	7	8#	12	26/ET
Medication Dispensation		X	X	X	X	X	X	X	X			
Estimated Visit Length (hours) →	2-3	1.5	.75	.75	.75	1.5	.75	.75	.75	1.5	1	1.5

SC=Screening/Assessment, EOT=End of Treatment, FU=Follow-Up, ET=Early Termination

ø the Week 0 visit is the Randomization Visit; ‡ individuals assigned female at birth only; * as-needed for safety/precautionary measure; ^ urine drug test should be performed at screening/baseline and again before randomization to ensure eligibility; $ should be completed prior to randomization to ensure eligibility; & if the Week 4 visit is missed, assessments that are only completed at that visit should be performed at the next attended visit, as long as the window for the Week 8 visit has not yet opened; # if the Week 8 visit is missed, assessments that are only completed at that visit should be performed at the next attended visit, as long as the window for the Week 26 visit has not yet opened; % these assessments may be performed at the randomization visit, as long as they occur prior to randomization to ensure eligibility; # training/practice cognitive task performance session; [!] data sharing of completed protocol procedures from PRO#54499 as a result of screen failure; > if ET occurs during treatment phase; [x] completed as part of data sharing with PRO#94743; [⊕] for visits occurring entirely remotely, will be collected and tested remotely by the participant, with results verified by video and photo capture

11.0 Specimen Collection and Banking (if applicable)

Urine specimens will be collected for laboratory testing, including drug tests, pregnancy tests, and ethyl glucuronide (alcohol use biomarker) tests. For visits conducted entirely remotely, the participant will collect and test urine specimens with CLIA waived qualitative ethyl glucuronide (EtG) tests, UDS, and pregnancy tests for participants assigned female at birth, with results verified using video or photo capture. Blood will be collected for laboratory testing, including carbohydrate deficient transferrin and phosphatidylethanol (alcohol use biomarker) tests. No samples will be banked after the aforementioned assays are completed. All laboratory testing will occur at the Clinical Neurobiology Laboratory in the Institute of Psychiatry on the MUSC campus. For visits conducted entirely remotely, blood will not be collected for laboratory testing.

12.0 Data Management

Statistical Methods

Power and Sample Size

This Phase II proof-of-concept trial is powered on the primary hypothesis that there will be a clinically significant differential in the decrease in total standard alcohol drinks and drinks per drinking day during the final four weeks of treatment (weeks 5-8) in the NAC group compared to the placebo group. In the proposed study, the two treatment arms, NAC and placebo, will be assessed for differences in drinking behavior using general linear models. In a prior pilot trial assessing adolescent drinking behavior (N=11), our team noted an average total of 49 (SD=26) drinks with 5.5 (2.0) drinks per drinking day during the 4 weeks prior to study enrollment. Additionally, although the participants noted in our prior CUD trial were not required to exhibit AUD nor was treatment targeted at drinking behavior, the heavier drinkers in the study (upper quartile of total drinks prior to baseline, ≥ 24 drinks/30 days) reported a mean of 58 (SD=30; median=54 drinks) drinks during the 4 weeks prior to study entry.[26] In this sample of heavier drinkers, those randomized to placebo had a median 10.7% decrease in total drinks at the end of CUD treatment (last 4 weeks of study treatment) as compared to a median 33.0% decrease in total drinks in the NAC treated group. Assuming a more robust treatment effect when alcohol use is the therapeutic target, a 50% decrease in total drinks during the final 4 weeks of study treatment in the active treatment group, as compared to baseline, would be a clinically relevant treatment effect. We conservatively estimate a similarly more robust 20% decrease in total drinks in the placebo treated group. Assuming similar baseline drinking behavior to the adolescent study (baseline mean=49 drinks/4 weeks) and a similar group level variance estimate as in the baseline study data (SD=25), we will have 80% power with a 5% type I error rate to detect the clinically relevant difference between a 50% decrease in the NAC treated group and 20% decrease in the placebo treated group (equivalent to 25 total drinks versus 40 total drinks during the final 4 weeks of treatment) with 45 participants randomized to each treatment group (n=90 total). Additionally, we anticipate that retention may be a challenge in this population, and we anticipate a 25% drop-out rate during study treatment, inflating the necessary randomized sample size to n=60 participants per treatment arm (**N=120 total randomized participants**). The noted sample will have greater than 90% power to find a

corresponding 50% decrease in drinks per drinking day in the NAC treated group as compared to a 20% decrease in the placebo treated group [2.8 (SD=2.0) vs. 4.4 (2.0) drinks per drinking day].

Primary Data Analysis

Baseline clinical and demographic characteristics will be collected and contrasts of baseline characteristics and clinical predictors of substance use will be performed between groups. Continuous and ordinal characteristics will be compared using a Wilcoxon Rank-Sum test statistic while categorical characteristics will be compared using a Pearson Chi-Square test statistic. If a baseline characteristic is significantly associated with the outcomes of interest, the corresponding variables will be used as initial covariates in the model analyses. Secondarily, possible covariates will be tested for confounding effects on the models specified and when present, they will be included in the final adjusted model. **Specific Aim 1:** The primary measure of interest will be the estimated reduction in self-reported standard alcohol drinks and the associated variance around these estimates during the final four weeks of study treatment (weeks 5-8) in those randomized to NAC as compared to those randomized to placebo. The main effect of NAC versus placebo on the total number of standard drinks will be assessed using generalized linear models and model-based means with associated standard errors will be reported. Analytic models will be computed both unadjusted and adjusted for significant covariates and confounders found in the preliminary analysis. **Specific Aim 2:** Self-reported drinking (weekly TLFB and mobile technology-delivered daily diary) as well as biomarkers of recent alcohol use (EtG, CDT, PEth, and alcohol breathalyzer) will be collected at study visits. Agreement between collection measures will be measured as percent agreement and kappa coefficients. Agreement will be assessed weekly during the entire time course of the treatment study and deviations between self-reported moderate/heavy use and biomarker-indicated moderate/heavy use will be assessed to characterize the reporting (under-reporting) of self-reported use amounts. Similarly, medication adherence will be measured at weekly study visits using self-report (self-report and mobile technology delivered video) and MEMS. Concordance percentages and kappa coefficients will be calculated between the adherence measurement methods. Due to high prevalence of self-reported adherence in substance use disorder studies, kappa coefficients will be calculated as the prevalence adjusted bias adjusted kappa (PABAK).[62] Clustered logistic regression model using the methods of generalized estimating equations will be applied to assess the differences in self-reported drinking and medication adherence measurement methods on binary adherence outcomes over time.[63] Working correlation structures will be independently compared and the final model structures will be chosen using the quasilikelihood under the independence model criterion statistic.[64] The main effects of measurement method and visit, as well as the interaction between method and visit, will examined for significance.

All randomized participants will be included in the primary analysis (intent-to-treat; ITT) and all statistical models will be implemented using SAS v. 9.4.

Secondary Data Analysis

In addition to the primary use outcome, secondary models will assess overall alcohol abstinence, the proportion of drinking days and the proportion of heavy drinking days noted during the final 4 weeks of study treatment. The temporal pattern in weekly drinking behavior over the course of the entire study is also of interest. Weekly drinking data in this population is likely to follow a Poisson distribution and will be assessed using random effects Poisson/Negative Binomial models and in the case of a high number of participants achieving abstinence from weekly alcohol use, zero inflated models will be explored. Similarly, binary weekly abstinence data will be assessed using mixed effects logistic regression models to assess the log-odds of weekly abstinence in the NAC- versus-placebo treated participants. Durability of treatment effects will be assessed through analysis of post-treatment alcohol use data at the Week 12 and Week 26 post-treatment visits, using model development processes similar to Specific Aim 1.

To assess the association between alcohol use reduction and changes in functional outcomes (alcohol-related problems; alcohol craving; cognitive task performance; anxiety, mood, and sleep quality measures; quality of life), general linear regression models will be developed. Outcome specific distributions will be used to model functional and cognitive outcomes (e.g., count, binary and Gaussian). For Gaussian

distribution assumptions, residual normality will be assessed using QQ plots, and when deviations from normality occur appropriate transformations will be made.

Missing Data and Attrition

Missing data are common in longitudinal alcohol clinical trials where use rates are the primary end point. The primary cause attributed to such missing data is participant attrition. Attrition can introduce bias into the parameter estimate and reduce study power, estimate precision and generalizability. In order to minimize missing data and study attrition, study simplification and enhanced communication between study coordinators and participants will be emphasized. We will make every effort to prevent attrition, e.g., phone/text/email reminders prior to visits, compensation for participation, meeting with participants in community if needed, and reinforcing adherence at each visit. However, these methods do not ensure that all data will be collected and appropriate analysis methods will be employed to accommodate missing data. Simply replacing missing data with the last observation carried forward or using worst case scenario adds substantial bias to both parameter and variance estimates.[65,66] Maximum likelihood methods (ML) yield valid inferences assuming ignorable attrition (i.e., attrition is accounted for by covariates or the dependent variable measured prior to dropout). Additionally, Full Information Maximum Likelihood methods (FIML) will be implemented and compared to the results from the ML estimation to assess bias that may be present due to missing data.[67] In addition, in keeping with the Intent to Treat Principle, we will make every effort to continue assessments for the entire course of randomized treatment, even among those who fail to adhere to randomized assignment or stop participating in the study assigned intervention.

13.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

Trial Management

All aspects of the study will be run through the MUSC Department of Psychiatry and Behavioral Sciences, where the MPI hold faculty appointments. The timetable is as follows:

	Year 1 (months)	**Year 2** (months)	**Year 3** (months)	**Year 4** (months)	**Year 5** (months)
Refine all procedures	1-3				
Procure supplies	1-3				
Refine recruitment methods	1-3				
Train Personnel	1-3				
Study Enrollment					
Cumulative *N* to enroll*	(22)	(30)	(30)	(30)	(8)
First Participant Enrolls	4				
First Participant Completes	10				
Last Participant Enrolls					51
Last Participant Completes					57
Data Analysis					58-60
Manuscript Preparation					58-60

All numbers reflect months within total study duration (*with the exception of cumulative *N*)

Data Management and Analysis

Data will be collected by the appropriate individual (research assistant, PI, Co-I) using standardized paper forms and will only be identified with the study's ID of the participant. The codes linking the name of the participant to the study ID will be kept confidential in a secured cabinet by the PI. Collected forms will be securely transported to the PI's data entry center. Research assistants will enter data in REDCap (Research Electronic Data Capture), a secure, web-based application designed exclusively to support data capture for research studies. REDCap provides: 1) an intuitive interface for data entry (with data validation);

2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages (SPSS, SAS, Stata, R); 4) procedures for importing data from external sources; and 5) advanced features, such as branching logic and calculated fields. These procedures are effective in minimizing data entry errors (e.g., missing or errant data). When possible, participant direct entry into REDCap (rather than paper forms) will be utilized.

Quality Assurance

Accuracy and completeness of the data collected will be ensured by weekly review. The REDCap system does not accept outliers, illogical response patterns, etc. The MPI and program coordinator will have weekly meetings with the research assistants to discuss qualitative comments received during data collection and any problems in data collection or entry. The statistician will periodically examine the database for potential irregularities. Initial data analyses will examine distributions of variable scores and comparability of baseline characteristics across conditions in case analyses need to be adjusted for these. Confidentiality protections are outlined above.

Regulatory Issues

Prior to the start of the study, the protocol will be registered on the clinical trials registry (clinicaltrials.gov). All serious adverse events will be reported to the MUSC Institutional Review Board (IRB) within 24 hrs. Follow-up of all serious adverse events will be reported as well. All adverse events are reviewed weekly by the MPI and yearly by both the DSMB and the IRB. Any significant actions taken by the local IRB, including significant protocol changes, will be relayed to NIAAA. We anticipate the serious adverse event rate to be extremely low. If monitoring indicates otherwise, we will convene a special meeting of the Data and Safety Monitoring Board (DSMB).

Trial Safety

The potential risks and benefits and methods to minimize these risks are outlined in **15.0 Risks to Subjects**. Guidelines have been developed for managing and reporting of adverse events (AEs), including serious adverse events (SAE; defined as any untoward medical occurrence that results in death, is life-threatening, requires or prolongs hospitalization, causes persistent or significant disability/incapacity, results in congenital anomalies/birth defects, or in the opinion of the investigators represents other significant hazards or potentially serious harm to research subjects or others). PI Dr. Gray will serve as the Program Manager for AEs. The Adverse Event Log will be used to document all AEs. If an AE is non-serious (self-limited with no intervention needed), no further action will be necessary. However, in the case of a serious, unresolved event, an AE follow-up log will be completed. The clinician will then call Dr. Gray with initial reports within 24 hours of the start of the SAE. The clinician will record the information on SAE Notification Form. The clinician will forward hard copies of the complete report (SAE Notification Form, Concomitant Medication Log, and AE Log) to Dr. Gray, who will, in turn notify the IRB, DSMB, and NIH about the SAE. Additionally, Dr. Gray will communicate summary reports of DSMB discussion of the SAE, or any deliberations of IRB regarding the review of the SAE or the trial itself, to NIH. If the event is "Serious, Unexpected and Associated" (an SAE is considered unexpected if it is not described in the Package Insert), Dr. Gray will complete Food and Drug Administration (FDA) Form 3500A and will forward it to the FDA. Dr. Gray also will inform the IRB and the study participants (and parents/guardians, as appropriate) about the SAE. In all of these reviews and reports, strict patient confidentiality will be maintained.

AEs will be coded on a weekly basis using Medical Dictionary for Regulatory Activities (MedDRA) rules and entered into a database. For each weekly study meeting, the research assistants will prepare a summary of all AEs, including their severity and presumed relation to study medication. The PI will review this at the weekly study meeting (or before if more urgent).

Study procedures will follow as much as possible the FDA's Good Clinical Practice Guidelines. We will encourage participants (and parents/guardians as appropriate) to notify their physicians that a) they are in a randomized controlled research study evaluating *N*-acetylcysteine for adolescent alcohol use disorder, and b) the physician should contact the PI directly if the physician has any questions.

The research assistants will be instructed not to reveal whether a person is a participant in the study and will report to the PI any outside requests for information about a participant or any breaches in confidentiality. All requests by participants' physicians and other medical providers will be referred directly to the PI.

Trial Efficacy

The Data and Safety Monitoring Board may request a blinded interim efficacy report (blinded to the PI and research team) for review while the trial is ongoing. Final (fully unblinded) efficacy analysis will occur after all participants have completed all visits.

Data and Safety Monitoring Plan Administration

The PI will be responsible for monitoring the trial. The statistician will examine the outcomes database quarterly for missing data, unexpected distributions or responses, and outliers. The PI will weekly check the adverse event database prepared by the research assistants immediately prior to the lab meeting to a) see if any particular MedDRA categories are being endorsed more frequently than anticipated, and b) determine if any adverse event symptom checklist scores are higher than expected. A DSM report will be filed with the IRB and NIDA on a yearly basis, unless greater than expected problems occur. The report will include participant characteristics, retention and disposition of study participants, quality assurance issues and reports of adverse events, significant/unexpected adverse events and serious adverse events. We will report efficacy at the end of the trial.

Data and Safety Monitoring Board

We will create a Data and Safety Monitoring Board, comprised of multidisciplinary faculty with expertise in pharmacotherapy and behavioral treatment trials (adolescent and/or addiction-related). The DSMB will meet annually (more frequently as needed for emergent situations) to review any AEs related to the study, as well as review any data management related errors. The board may be called at any point if needed for serious adverse events, etc. Modification will be made in the procedures and/or the protocol if necessary based on the findings of the board.

14.0 Withdrawal of Subjects (if applicable)

Discontinuation/Stopping Rules

Every effort will be made to retain participants in the trial. If a participant experiences intolerable adverse effects with study medication that are not remedied by a dose reduction, the medication may be discontinued while the participant continues to participate in all non-medication study interventions and procedures.

Clinical Deterioration "Rescue" Plan

A clinical deterioration "rescue" plan will be in place for participants that experience psychiatric or substance use deterioration during the study. Symptoms will be monitored closely throughout the trial to assess for deterioration and appropriate intervention will be arranged for any participant demonstrating gross clinical deterioration. The rescue measures will include immediate assessment by the medical clinician for a comprehensive psychiatric and substance abuse evaluation and referral for appropriate clinical intervention. MPI Gray has full medical and psychiatric admitting privileges at the MUSC Medical Center.

Referral for Participants Needing Continuing Treatment

At the end of study participation, if a participant requires or requests continuing treatment for AUD, an appropriate treatment referral will be made.

15.0 Risks to Subjects

Potential Risks

Questionnaires and interviews are all non-invasive and, as such, involve minimal physical risk to participants. Potential risks incurred by participants include:

1. Adverse events related to study medication
2. Loss of confidentiality
3. Venipuncture-associated risks

Adverse events related to study medication

N-acetylcysteine has a generally benign adverse effect profile. A meta-analysis of studies evaluating long-term oral treatment with *N*-acetylcysteine for prevention of chronic bronchitis found that *N*-acetylcysteine was well tolerated, with generally mild, most commonly gastrointestinal, adverse effects that did not require treatment interruption.[17] Our experience to date with *N*-acetylcysteine at the same dose proposed in this trial in both adolescents and adults with substance use disorders, many of whom used alcohol frequently, suggests a benign adverse event profile.[19-26]

Some patients who have taken intravenous *N*-acetylcysteine for the treatment of acetaminophen overdose have had more serious reactions. Allergic reactions have occurred in about 5% of patients taking intravenous *N*-acetylcysteine.[68] These reactions may be mild, consisting of flushing, rash, and itching. Less common side effects include trouble breathing, low or high blood pressure, fever, and hives. If untreated, such a reaction could lead to death. Even more rare serious side effects of intravenous *N*-acetylcysteine are irritability, confusion, and seizures. These reactions (severe allergic reaction or seizures) have never been reported when *N*-acetylcysteine is taken orally, as it will be in this study.

Loss of confidentiality

Any communication of personal information carries the potential risk of breach of confidentiality.

Venipuncture-associated risks

The risks of drawing blood include temporary discomfort from the needle stick and bruising. This risk is not relevant to remote-only participants.

Adequacy of Protection Against Risks

Recruitment and Informed Consent

Recruitment of the participants will be from the local community. The Medical University of South Carolina (MUSC) Institutional Review Board (IRB) approved Informed Consent (IC) will be obtained prior to the start of any study procedures as part of the initial assessment. The consent will be explained orally and in the written form, and will be documented by the signature of the participant on the IC. For participants under 18 years old, a parent/legal guardian will provide consent and the participant will provide assent. The consent document will contain a thorough review of potential risks associated with trial participation, including potential medication-related risks.

Absence of Coercion: Participation in the study is voluntary. Compensation for study participation is consistent with the level of burden involved in completing study procedures, and is consistent with the compensation schedule in similar prior studies. The informed consent agreement that will be read to each volunteer (and parent/guardian as applicable) prior to enrollment in the study explains the following:

a) Compensation is supplied at each study visit.

b) Participants may discontinue participation in the study at any point.

c) Withdrawing from the study will not result in any adverse consequences to the participants.

Protection Against Risk

Adverse events related to study medication

The informed consent process will be used to thoroughly educate participants and parents/guardians about potential medication-related risks, including adverse events. This discussion will include thorough review of adverse events associated with oral *N*-acetylcysteine treatment. Rigorous screening procedures and strict exclusion criteria are designed to exclude potential participants at elevated risk for adverse events. The study medical clinician will conduct serial adverse events monitoring as part of medication management. Participants and parents/guardians will have access to the study medical clinician 24 hours, 7 days a week for emergencies. Participants experiencing intolerable adverse events will have the opportunity to reduce dose or discontinue medication altogether, while remaining in the study for ongoing monitoring. MPI Gray has full hospital admitting privileges in the event of an adverse event requiring hospitalization. Urine pregnancy tests will be conducted at baseline and serially during treatment for participants assigned female at birth.

Loss of confidentiality

The research team has established procedures in place to minimize the risk of any confidentiality breach. Participant records are stored in locked files within locked offices that are locked during holidays, weekends, and non-working hours. Information contained in computer databases is password protected, maintained by participant number only, and devoid of specific identifiers. No specific or general participant information will be left in public access areas, and no oral communication regarding participants with identifiers will be made in any public areas. Research staff members have had extensive training in HIPAA regulations and in maintaining confidentiality.

Venipuncture-associated risks

The blood draws for alcohol use biomarker testing will be conducted by an experienced phlebotomist with all required training and certification. Any concerns or issues related to the blood draw will be addressed as needed.

16.0 Potential Benefits to Subjects or Others

Participants in this study, regardless of randomization to active or placebo medication, may benefit by receiving a) comprehensive medical and psychiatric evaluation, and b) brief alcohol intervention throughout active treatment.

The potential benefits of the knowledge to be gained from the proposed study are considerable. The proposed study will answer important questions regarding the potential of NAC as a pharmacotherapy for adolescent AUD, while also evaluating developmentally-informed methods to guide future pharmacotherapy trials for adolescent AUD. The plans for monitoring risk as described above warrant the conduct of this study for the knowledge that may reasonably be expected to result.

17.0 Sharing of Results with Subjects

At the conclusion of study participant procedures (after the last data point is entered for the last participant), the randomization assignments will be unblinded. Participants may contact the research team at that point to learn their treatment assignments (i.e., NAC or placebo). The research team will also welcome inquiries from participants on the overall results of the trial, which will also be provided via scientific publication and posting to clinicaltrials.gov.

18.0 Drugs or Devices

NAC and placebo capsules will be compounded and dispensed by the MUSC Investigational Drug Service, using established methods across multiple other NAC trials.

The Principal Investigator (Dr. Gray) holds an IND for NAC, and has submitted an IND amendment request to add this protocol.

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