Identifying Biological Signatures of N-Acetylcysteine for Non-Suicidal Self-Injury in Adolescents and Young Adults

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Tool Revision History

Version Number: 3
Version Date: 01 March 2019
Summary of Revisions Made: Transfer to NCCIH template

Version Number: 3.2
Version Date: 08 May 2019
Summary of Revisions Made: Response to NCCIH comments; shift baseline measures from Day 0 to Day 1 to standardize time length from baseline to Day 28; administrative changes

Version Number: 3.3
Version Date: 25 July 2019
Summary of Revisions Made: Added Minnesota State Fair recruitment booth information to recruitment section of protocol

Version Number: 3.4
Version Date: 24 October 2019
Summary of Revisions Made: Response to site initiation visit comments; removed bioavailability as a study objective; changed procedure for MRI toxicology screen to reflect that participants will still be scanned if results are positive; added new measure to track participants' ongoing medication use and changes during the study; added remote completion option for REDCap self-report online questionnaires; added optional text communication; added REDCap survey as an option for medication adherence tracking; added recruitment from clinic; changed personnel.

Version Number: 4
Version Date: 25 February 2020
Summary of Revisions Made: Study drug manufacturer was changed from BioAdvantex to Swanson Health Products. The study drug administration was revised to reflect this change. The quantity of drug per pill reduced from 900 mg/tablet to 600 mg/capsules, and the method of ingesting the drug was changed from dissolving tablets to swallowing capsules. Added Cash Choice Task, added new location (the Research in Adolescent Depression Lab at the University of Minnesota Medical School) for the Day 0 (intake) visit, and Day 14 (mid-study) visit, and added explanation regarding CMRR Subject Information form.

Version Number: 5
Version Date: 10 August 2020
Summary of Revisions Made: Added COVID safety screen and other safety practices as outlined in the sunrise plan by PI Dr. Cullen for all in-person visits. Added option of video conferencing for visit 1 and visit 3. Changed the plan for study drug supply to the participant. Now a 4-week supply of the study drug will be given to the participant on visit 2. Added abbreviated PK sample collection scheme for visit 4 (day 28) to be used during the COVID-19 pandemic. Added participants' choice of multiple needle sticks or placement of IV catheter on visit 4 when practicing abbreviated PK sample collection scheme.

Version Number: 6
Version Date: 18 September 2020
Summary of Revisions Made: Replaced consenting language to reflect template for e-consenting procedures. Corrected a discrepancy in consenting procedural description to reflect transition to e-consenting vs. in-person consenting procedures.

Version Number: 7
Version Date: 10 December 2020
Summary of Revisions Made: Moved BDI-II assessment to Study Day 0 (Consenting Visit) in order to obtain necessary randomization characteristics pre-MRI scanning on Day 1.

Version Number:8
Version date: 21 February 2021
Summary of revisions: Added provision of partial compensation to participants based on the tasks completed in the study/visit, to accommodate scenarios where participants only complete a fraction of the planned activities.

Version Number: 9
Version date: 22 March 2021
Summary of revisions: Clarified eligibility language regarding “significant tissue damage” in order to use the same language between protocol and screening forms, moved SITBI to Day 0 activities in order to better inform eligibility determination, developed an online self-report REDCap survey for participant screening.

Version Number: 10
Version date: 05 May 2021
Summary of revisions: Assigned the WASI-II to be completed on Day 1 *or* 28. Remove BSS on Day 0 (only collect on Day 1 and 28) to limit patient burden. Added additional warning re: blood draw risks (fainting and/or dizziness) and additional common side effects of NAC (rash and itchiness). Removed language describing “medication boxes,” as study medications are provided in bagged, individual RX bottles at this time. Added language to specify that medication tracking diaries are only provided on Days 1 & 14 if the hardcopy option is elected by the participant. Changed final visit window from “28 +/- 3 dayfs” to “28-32 days,” to accurately reflect supply provided by IDS since COVID protocol changes. Additionally added flexibility to fill scripts up to 35 days if a participant is unable to complete final visit during 28-32 day medication supply window. Added detailed language regarding reporting of protocol deviations (per Advarra Handbook).

Version Number: 11
Version date: 01 July 2021
Summary of revisions:
(1) Clarified language describing more specifically when a participant may be withdrawn from the study due to suicidal ideation, moved text on study withdrawal from Section “6.2.5 Final evaluation” to Section “8. Intervention Discontinuation”, and edited that section for clarity.
(2) Added the role of an Independent Clinical Monitor, described in Safety sections 7.1 – 7.6 .

(3) Removed the SITBI on Visit 4 to reduce participant burden.
(4) Broadened inclusion /exclusion criteria to allow mild Alcohol Use Disorder, mild Cannabis Use Disorder, and Substance Use or Alcohol Disorders in early remission (at least 3 months of abstinence).
(5) Clarified assessment of side effects and how they are evaluated as potential adverse events, added detail on monitoring procedure (emails sent to study team when triggered by threshold-level responses on key safety measures), and moved this text about side effects assessment from Section “6.2.4 Follow-up Visits” to Sections “7.1 Specification of Safety Parameters” and 7.2 “Methods and Timing for Assessing, Recording and Analyzing Safety Measures”.
(6) Clarified 14-day pill count (will now be done by participant over Zoom on 14-day visit).
(7) Deleted duplicated paragraph beginning with “Adverse Events, Unanticipated Problems and Serious Adverse Events”.
(8) Added the definition of Unanticipated Problem.
(9) Clarified language describing consent process to include dual authentication.

Version Number: 12
Version date: 06 October 2021
Summary of revisions: Added language to describe the expected number of people to be consented and changed the expected number of participants to be enrolled from 45 to 46.

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PARTICIPATING STUDY SITES

University of Minnesota, Department of Psychiatry and Behavioral Sciences

PRÉCIS

Study Title

Identifying Biological Signatures of N-Acetylcysteine for Non-Suicidal Self-Injury in Adolescents and Young Adults

Objectives

Primary: To measure NAC-induced changes to concentrations of glutathione (GSH) in the anterior cingulate cortex (ACC) as measured by magnetic resonance spectroscopy (MRS) in 36 adolescents and young adults with NSSI (12 in each group: high, low, and placebo). Secondary:

1) Measure NAC-induced changes in the reduced-to-oxidized ratio of GSH (GSH/GSSG) in blood
2) Measure the NAC-induced changes to concentrations of glutamate (GLU) in the ACC as measured by MRS
3) Measure and compare tolerability of high dose NAC, low dose NAC and placebo
4) Characterization of NAC pharmacokinetics
5) Measure NAC induced biomarker changes in levels of the antioxidant proteins heme oxygenase-1 and catalase in blood.
6) Measure concentrations of GABA in the ACC by MRS.
7) Measure NAC induced biomarker changes in resting-state functional connectivity between amygdala and insula.

Design and Outcomes

This study is a double-blind, placebo controlled, 4-week course of two-tiered N-acetylcysteine (NAC) dosing focused on identifying the optimal dose to achieve meaningful change in measurable biomarkers (glutathione and glutamate).

This design will allow us to confirm acute biological changes, select the optimal dose for achieving biological effects, and examine dose/concentration-response relationships with respect to biological markers and pharmacokinetics.

Brief schedule of activities: Subjects will be recruited through community and clinical settings and screened using an online form. There will be a total of 4 in-person visits and two sets of on-line study activities.

- Day 0 -- Visit 1: Consent, clinical assessment, cognitive testing, randomization / group assignment
- Day 1 -- Visit 2: baseline measures, MRI scan, blood draw and begin study

medication

- Day 7: At-home online surveys
- Day 14: Visit 3: In-person visit, re-supply NAC/PBO
- Day 21: At-home online surveys
- Day 28 -- Visit 4 (final visit): NAC/PBO final dose, MRI scan, 5 blood samples via intravenous (IV) catheter for pharmacokinetic (PK) analyses, clinical assessment

Interventions and Duration

Eligible participants will be assigned to one of 3 groups (double-blinded): a low-dose NAC group (3600 mg/day), a high-dose NAC group (5400mg/day), and placebo (PBO). The study intervention period is 4 weeks. Total participation is up to 8 weeks, depending on the length of time between Day 0 and Day 1.

Sample Size and Population

We will recruit 36 adolescents and young adults aged 16-24 years. There will be 12 participants in each group (PBO, 3600mg/day, 5400mg/day). We will use a minimization procedure to ensure that the participants in these 3 groups will have similar age, clinical severity and medication status.

1. STUDY OBJECTIVES

1.1 Primary Objective

To test the hypothesis that oral NAC treatment (but not placebo) will lead to at least a 5% increase in glutathione (GSH) concentrations in the anterior cingulate cortex as measured by magnetic resonance spectroscopy (MRS) in a study of 36 adolescents and young adults with NSSI.

We will test two NAC doses (3600mg and 5400mg). Dose superiority will be defined both by change in GSH and by tolerability. Specifically, the superior dose will be the one which showed capability of achieving the greatest increase in GSH in the ACC as measured by MRS, and where no more than 20% of subjects at that dose discontinue or diminish their dose due to medication adverse effects (See section 5.1 for adverse effects and diminishing dose). (In other words, no less than 80% of subjects at a dose can discontinue or diminish their dose due to adverse effects in order to qualify as a superior dose.)

1.2 Secondary Objectives

1) Test the hypothesis that oral NAC will lead to an increase of at least 50% (degree of change will be greatest for NAC 5400mg, lesser extent for NAC 3600mg, minimal / no change for placebo) in the reduced-to-oxidized ratio of GSH (GSH/GSSG) in blood.
2) Test the hypothesis that oral NAC (but not placebo) will lead to a decrease of

at least 5% (degree of change will be greatest for NAC 5400mg, lesser extent for NAC 3600mg, minimal / no change for placebo) in the concentrations of glutamate (GLU) in the ACC as measured by MRS.

3) To measure tolerability of high dose NAC, low dose NAC and placebo
4) Characterization of NAC pharmacokinetics
5) To measure additional NAC-induced biomarker changes (degree of change will be greatest for NAC 5400mg, lesser extent for NAC 3600mg, minimal / no change for placebo) in blood sampling of antioxidant protein levels (catalase and heme oxygenase-1).
6) To measure additional NAC-induced biomarker changes in the concentrations of GABA in the ACC
7) To measure additional NAC-induced biomarker changes (degree of change will be greatest for NAC 5400mg, lesser extent for NAC 3600mg, minimal / no change for placebo) in brain imaging (resting-state functional connectivity between amygdala and insula).

2. BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

Non-suicidal self-injury (NSSI), the deliberate act of damaging one's own tissues without suicidal intent (Winchel and Stanley 1991). Onset of NSSI typically occurs in early-mid adolescence (Andover 2014; Andrews et al. 2013) and adolescent NSSI has an international prevalence of approximately 18% (Muehlenkamp et al. 2012). NSSI has been shown to predict negative outcomes such as persistent psychopathology and suicide attempts (Horwitz, Czyz, and King 2015; Tang et al. 2011; Victor and Klonsky 2014). Treatments for this habitual, self-destructive behavior pattern are sorely limited (Brent et al. 2013; Hawton et al. 2015). Since adolescence is notable for ongoing brain development (Giedd et al. 1999), it represents a critical window of opportunity to derail dangerous behavior trajectories, restore healthy behavior, and prevent negative outcomes (Paus, Keshavan, and Giedd 2008). Identification of novel, biologically-informed treatments for adolescent NSSI could improve health outcomes over the lifespan.

2.2 Study Rationale

N-acetylcysteine (NAC), a dietary supplement that is widely-available over-the-counter and by prescription, may have promise as a novel treatment for adolescent NSSI. NAC is the N-acetyl derivative of the amino acid L-cysteine. It can be found in health food stores with other natural products and also is a prescribed drug for conditions including Tylenol overdose and certain respiratory diseases. The pharmacokinetic (PK) properties of oral NAC have already been established in humans (Holdiness 1991). Several reviews have highlighted a growing literature suggesting that NAC can be helpful for a range of psychiatric disorders (Berk et al.

2013; Ng et al. 2008; Deepmala et al. 2015).

We conducted an open-label pilot study showed a reduction of NSSI frequency in adolescents after 8 weeks of oral NAC (Cullen et al. 2018). These exciting findings prompted the proposed work taking next steps to investigate the possible use of this widely-available dietary supplement for a common and serious adolescent clinical problem.

In preparation for a definitive clinical NAC trial for adolescent NSSI, a critical preliminary step is to clarify NAC's biological signatures, or measures of the mechanisms underlying its clinical effects, to provide the basis for biologically-informed design of optimized efficacy trials. Literature to date (see review by (Berk et al. 2013) suggests two possible biological signatures for NAC's behavioral effects: (1) increasing glutathione (GSH), the primary antioxidant in the brain; and/or (2) modulating the glutamate (Glu)-cysteine antiporter (via cysteine [CYS], NAC's primary metabolite), effectively decreasing excessive Glu transmission. Both of these mechanisms could alleviate stress-induced neurotoxicities in adolescents with NSSI, but neither has been evaluated in NSSI. Based on current knowledge including our own past work, this study seeks to identify NAC's biological signatures in adolescents with NSSI, as follows:

1) Biological Signature #1: NAC increases GSH, a potent antioxidant. A key biological mechanism that has been proposed to underlie NAC's positive impact on mental health is via enhanced production of GSH, the primary antioxidant in the brain, providing a neuroprotective effect against the toxicities associated with stress (Berk et al. 2013; Ng et al. 2008). Oxidative stress mechanisms have been implicated in affective disorders (Siwek et al. 2013). In adolescents, NSSI behavior typically represents an attempt to self-regulate extreme levels of negative affect (Klonsky 2007). Furthermore, adolescents with NSSI frequently report a history of severe adverse experiences (Jacobson and Gould 2007) which themselves are known to have lasting adverse impacts on the CNS including neuronal damage (McEwen 2003; Goldwater et al. 2009; McEwen, Nasca, and Gray 2016; Lupien et al. 2009). Research in humans has shown that NAC preserves mitochondrial bioenergetics and normalizes GSH levels following spinal cord injury (Patel et al. 2014) and following brain injury (Pandya et al. 2014). A recent study of children with autism showed that NAC led to increased blood GSH levels compared to PBO (Wink et al. 2016). In a developmental animal model of schizophrenia (early hippocampal lesions in rats), NAC was shown to prevent brain and behavioral alterations induced by the lesions through diminishing oxidative stress (Cabungcal et al. 2014). In a mouse model of Parkinson's disease, mice with impaired cysteine uptake show evidence of oxidative stress and early cell death of dopaminergic neurons in the nigrostriatum; both processes were alleviated by NAC (Berman et al. 2011). Research in rodents has suggested that NAC leads to increased GSH redox ratios in brain tissue that persist after NAC is out of circulation (Zhou et al. 2015). Magnetic resonance spectroscopy (MRS) is capable of non-invasively measuring cortical GSH concentrations in-vivo, and systemic levels are typically

measured using the GSH redox ratio (GSH/GSSG). Despite the relevance of oxidative stress mechanisms to NSSI, no research to date has formally tested treatments targeting this system in adolescents with NSSI using appropriate methodology to confirm this potential biological signature.

2) Biological Signature #2: Glutamate (Glu) is the primary excitatory neurotransmitter in the brain, and excessive Glu signaling plays a key role in impaired synaptic plasticity and impaired regulatory control of impulses to engage in maladaptive behaviors (Kalivas and Volkow 2011, Brown et al. 1988). Since NSSI is a maladaptive, habitual behavior, it has conceptual overlap with other disorders along the impulsive/compulsive spectrum such as addiction, gambling, hair-pulling, skin-picking, and obsessive-compulsive disorder, in which dysregulation within the glutamate (Glu) system has been implicated (Kalivas and Volkow 2011). NAC regulates glutamate via the Glu-cysteine antiporter andrevious studies have demonstrated that NAC may alleviate these impulsive/compulsive problems (Rothbart et al. 2013; McClure et al. 2014; Grant et al. 2016; Brown et al. 1988). NAC's primary metabolite cysteine (CYS) gets converted to cystine, which then enters glial cells in exchange for Glu via the Glu-cystine antiporter. Pre-clinical research on cocaine dependence showed that by this mechanism, NAC leads to increased extracellular glutamate via exchange of extracellular cysteine with intracellular glutamate, ultimately pumping glutamate out of glial cells (Baker et al. 2003; Moussawi et al. 2011). Increased concentrations of glutamate in the extracellular compartment serves to inhibit glutamate transmission ultimately serving to decrease glutamatergic neurotransmission (Moran et al. 2005) MRS is capable of non-invasively measuring cortical Glu concentrations in-vivo. While MRS cannot distinguish intracellular from extracellular Glu, it primarily measures intracellular Glu (R. Gruetter et al. 1998) Previous MRS research in adults with cocaine dependence showed that NAC led to decreased Glu in the anterior cingulate cortex (ACC; (Schmaal et al. 2012), a region of the brain that is implicated in regulating emotion and controlling impulses (Bush, Luu, and Posner 2000). In summary, a potential biological signature for NAC is its modulation of the Glu-cystine antiporter, downregulating excessive glutamate transmission and associated excitotoxicity. This biological signature can be indexed by MRS: decreased Glu concentrations in the ACC would indicate reduction in intracellular Glu concentrations due to NAC's modulation of the Glu-cystine antiporter. However, although the Glu system has been hypothesized as an important potential mechanism underlying NAC's behavioral benefits and in particular may be highly relevant to perpetuating NSSI, no studies have yet attempted to probe this system in adolescents with NSSI.

Finally, confirmation of NAC's biological signatures is challenging due its complex pharmacokinetics (PK). Oral NAC is primarily used in psychiatric studies because of its ease of use, but has low bioavailability (5-10%). While multiple PBO-controlled clinical trials have shown that oral NAC, typically at modest doses such as 1200mg-3000mg/day, produces significant behavioral changes in several psychiatric disorders (Berk et al. 2013), to date there is a paucity of evidence demonstrating that oral NAC

leads to significant direct antioxidant effects in the human central nervous system. Mental health studies have generally not included PK and pharmacodynamic (PD) components-- a missed opportunity to explain mixed findings with respect to clinical efficacy.

Summary and Scientific Premise: Adolescent NSSI is a severe clinical problem in need of new treatments. Results from an open-label pilot study suggest that NAC may be clinically useful in reducing frequency of NSSI in adolescents. Next steps in investigating this potential treatment will require a clear understanding of NAC's biological signatures and how they relate to behavior change. Based on existing knowledge, two candidate biological signatures for NAC include (1) increasing GSH in the brain, thus serving as an antioxidant, and (2) modulating Glu, serving to reduce stress-induced excitotoxicity. The current proposal will investigate these biological signatures of oral NAC in adolescents with NSSI using a study design that will address current knowledge gaps regarding the dose required to achieve PK and PD responses. Oral administration is selected for this study because of its ease of use and broader applicability in clinical practice. The proposed NAC doses are selected based on (1) our prior pilot study in adolescents with NSSI showed clinical improvement following an 8-week course of treatment of oral NAC at a dose of 3600mg/day (Cullen 2018), and (2) our prior study in adults with neurological disease which showed changes in GSH following a 28-day course of treatment with oral NAC at a dose of 6000mg/day (Coles 2018). The 4-week period is selected to determine effects from acute oral NAC treatment on the brain, as was done in Coles et al 2018.

3. STUDY DESIGN

Overview: This study will investigate NAC's biological signatures in adolescent NSSI, testing whether a meaningful change in measurable biomarkers (GSH and GLU) can be achieved following a 4-week course of NAC treatment. We will recruit 36 adolescents and young adults aged 16-24 years. They will be assigned to one of 3 groups (double-blinded): a low-dose NAC group (3600 mg/day), a high-dose NAC group (5400mg/day), and PBO. This design will allow us to confirm acute biological changes, select the optimal dose for achieving biological effects, and examine dose/concentration-response relationships with respect to biological markers and PK.

Primary outcome measure:

1) GSH concentration in ACC.

Secondary outcome measures:

1) Reduced-to-oxidized GSH ratio in blood (GSH/GSSG)
2) Glu concentrations in ACC
3) Tolerability of NAC as measured using a side effects checklist
4) Pharmacokinetics of NAC and its metabolites, CYS and GSH
5) Antioxidant protein levels (catalase and heme oxygenase-1 [HO-1]) for which previous studies have shown increases following NAC treatment
6) GABA concentrations in ACC. Our pilot study found a significant increase in

GABA levels after 28 days of oral NAC (6000 mg/day) (Coles et al. 2018).

7) Functional connectivity between amygdala and insula. Our pilot study showed that after treatment with oral NAC (3600 mg/day), participants with NSSI showed an increase in amygdala functional connectivity in a cluster that included the bilateral insula.

Table 1 - Participant study duration:

Day 0 (in-person or video conference)	3 hours
Day 1 (in-person)	4 hours
Days 2-27 (at home)	Study drug taken at home (5 capsules in the morning, 4 capsules in the evening)
Days 7 and 21 (online forms at home)	0.5 hours
Day 14 (+/- 2 days) (in-person or video conference)	0.5 hours
Day 28 (+/- 3 days) (in-person)	3-8 hours (shorter protocol during COVID-19 pandemic)
Total	11-16 hours over 4 weeks

Duration to enroll all participants: 2 years

Duration to complete all study procedures, including analysis: 3 years

Study locations:

1) Ambulatory Research Center
   Department of Psychiatry and Behavioral Sciences
   University of Minnesota, Twin Cities Campus
   F212/2C West
   2450 Riverside Ave
   Minneapolis, MN 55454
2) Research in Adolescent Depression Lab (RAD Lab) @ Riverside Park Plaza
   University of Minnesota Medical School
   701 25th Ave S Suite #515,
   Minneapolis, MN 55454
3) Center for Magnetic Resonance Research
   Department of Radiology
   University of Minnesota, Twin Cities Campus
   2021 6th Street SE
   Minneapolis, MN 55455

Study intervention description: NAC is a natural product that may have promise for treating NSSI in adolescents. NAC is the N-acetyl derivative of the amino acid L-

cysteine and a precursor of the antioxidant glutathione. It can be found in health food stores with other nutritional and dietary supplements. The bioavailability and pharmacokinetic (PK) properties of intravenous (IV) and oral NAC have already been established in humans (Holdiness 1991).

Study intervention handling: The SW854 product will be provided to the Investigational Drug Service (IDS) Pharmacy by the company Swanson Health Products. UMN IDS will supply and prepare matching placebo, as well as handle all NAC and placebo allocation and dispensing. All participants, family members, and the entire research team will be blinded to active NAC versus PBO assignment. If there are any leftover capsules not taken by a participant, these will be returned to IDS, recorded and disposed of according to University of Minnesota IDS procedures.

Randomization and Double-Blind. Participants who meet criteria for entry into the study (based on the online screen and the clinical interview) and who consent to participate will be randomized to active NAC high dose vs. active NAC low dose versus PBO (1:1:1). We will implement an adaptive randomization procedure called minimization. The purpose of the procedure is to ensure that the 3 groups are similar with respect to key demographic and clinical variables (such as age, NSSI severity, medication use). The study statistician will use this participant information to assign a blinded randomization group (A, B, or C) using the minimization procedure.

The study statistician will then pass the blinded group assignment to the research pharmacy, IDS. Before the first participant is randomized, IDS will randomly assign "NAC high" "NAC low" and "PBO" to the letters A, B, and C; this assignment (of treatment name to treatment letter) will not be communicated to anyone outside of IDS until the study is complete and the study team is ready to break the blind. All participants, family members, and the entire research team (including the study statistician) will be blinded to high NAC versus low NAC versus PBO assignment. The IDS pharmacist will be authorized to break the blind on an individual participant's treatment assignment in the event of that participant's medical emergency. See Section 6.2.2 for additional details.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

Participants must meet all of the following inclusion criteria in order to participate in this study:

1) 16-24 years old
2) Female assigned at birth.
3) Current frequency of at least one NSSI episode in the past 2 months
4) ≥ 5 past episodes of NSSI with significant tissue damage (e.g. skin is broken or bruised, and/or scars resulted from the injury)

5) Psychotropic medications are dose-stable for 1 month.
6) Ability to understand study procedures and to comply with them for the entire length of the study.

4.2 Exclusion Criteria

All candidates meeting the following exclusion criteria will be excluded from study participation. Candidates will be screened for these criteria prior to consent. If it is found that participant meets exclusion criteria after consent (i.e. at Visit 1 Clinical Assessment) participants will be removed from the study or given the necessary time to meet eligibility before continuing with study activities.

1) Any MRI contraindications (e.g. metal plates, claustrophobia, braces, implanted devices)
2) Male assigned at birth.
3) Any current serious medical illness as defined by medical history
4) Current Substance Use Disorder (except Tobacco Use Disorder, mild cannabis use disorder, mild alcohol use disorder, or SUD in early remission with abstinence ≥3 months)
5) Primary psychotic disorder (e.g. schizophrenia, schizoaffective disorder, schizophreniform disorder)
6) Neurodevelopmental disorder such as mental retardation or autism
7) Changes in psychotropic medications in past 1 month
8) Taken NAC or glutathione on a regular basis in the past 6 months
9) Currently pregnant, planning to become pregnant, currently breastfeeding, or unwillingness to use contraception throughout the study.
10) Allergy/sensitivity to N-acetylcysteine.
11) Inability or unwillingness of individual or legal guardian/representative to give written informed consent.

Rationale: Males are excluded for two reasons. First, since there is significant sexual dimorphism of brain circuitry that emerges during the adolescent period, given the small sample, we want to minimize sex-related variability. Second, the prevalence of NSSI is higher in females. In the NAC/NSSI pilot study conducted by our lab, 40 females and 1 male enrolled. As a result, the single male needed to be excluded due to being the only one.

4.3 Study Enrollment Procedures

Our goal is to have completed data for analysis on 36 participants, with the expectation of most of these coming from the University of Minnesota Psychiatry Department research registry and Fairview in-patient units (referrals and flyers). Participants will also be recruited within the local community using flyers and social

media marketing. We expect to consent up to 80 people and enroll up to 46 participants with the expectation of up to 20% attrition due to participant withdrawal.

Recruitment

We will pursue the following procedures for candidate recruitment:

1) We will contact participants from our prior research outreach efforts who were either ineligible, screened out or participated in previous research, and who had indicated a willingness to be contacted in the future.
2) We will use the research participant registry based in the University of Minnesota Psychiatry outpatient clinic.
3) We will utilize the M Health clinical data repository of those Fairview patients elected to participate in University of Minnesota research. Fairview Research Services provides a service that identifies patients in this database that fit eligibility criteria and sends a letter to the patient informing them about the research opportunity.
4) We will screen electronic medical records of adolescents with a history of self-injurious behavior treated in the clinics and hospital services of the University of Minnesota, Medical Center and Masonic Children's Hospital. We will only look at the records of those patients who have not opted-out of having their medical records used for research on the Consent for Services form they complete at intake. When potential cases are identified, we will contact the treating physician to discuss the patient and whether they might be suitable for consideration for the study. If yes, we will ask the physician (or they physician may choose to delegate this to another member of the treatment team) to approach the parent or guardian about the study. The physician (or delegated treatment team member) will let parents know that their daughter may be eligible to participate in a research study being conducted at the University of Minnesota, and will ask the parent or guardian whether they would like to be contacted by the research team to learn more about the study. The physician (or delegated treatment team member) will explain that participation is completely voluntary and in no way related to the treatment for their daughter. Information about study opportunities will also be provided for potential participants on a research board on the unit. The board will (a) post information about the study, (b) provide small cards with study information that parents can take home.
5) We will use the Fairview research recruitment mailing process. Potential participants are identified, based on protocol eligibility criteria, from a clinical data repository that houses the records of over 2 million patients. Once

identified, Fairview mails the IRB approved study recruitment letter to its patients on behalf of the researcher.

6) We will post advertisements in the community via flyers and local newspapers. We will use a Quick Response (QR) code on all printed and digital materials which will direct potential participants to a REDCap survey The REDCap survey asks for contact information, birth date of the potential participant, and also if they have ever self-injured. The REDCap survey and responses are to be maintained by the study team.

7) We will advertise through the internet, social media (Facebook, Pandora, Tumblr, etc.) and community outlets about our study and create an informative space about research opportunities and information about depression, self-harm and mental health for adolescents and young adults. Social media accounts for this research lab (RAD Lab) will be used for this study, as well as accounts for the University of Minnesota Department of Psychiatry managed by the Research Recruitment Specialist working on behalf of research staff for recruitment. Social media and internet traffic will be directed to a HIPAA compliant REDCap screening form (survey) on the website of our research lab (radlab.umn.edu) which will capture screening information for self-selected study candidates interested in more information and/or participation.

8) We will use advertisements on digital media platforms to reach our research population via demographic and key words. An interested individual's click will bring them to a REDCap survey. The REDCap survey asks for contact information, birth date of the potential participant, and also if they have recently self-harmed.

9) We will meet regularly with clinical groups affiliated with the University of Minnesota that are involved in the treatment of the target population to build awareness of the study and enhance referrals.

10) We will maintain a social media presence to promote the study and enhance visibility to the community. A coordinating website "radlab.umn.edu" will be used to describe participation information, including inclusion and exclusion criteria and amount of time to complete study. This information will also be posted on the University of Minnesota Department of Psychiatry website "z.umn.edu/findastudy". The website and media pages will provide study team contact information and IRB approval number. Media sites will be compliant with HIPAA: no identifying information will be collected and potential participants will be advised to contact the research team directly via phone or email for additional information about this study. The lab will include the following disclosure: "Disclaimer: The information on this site is

not intended or implied to be a substitute for professional medical advice, diagnosis or treatment. All content, including text, graphics, images and information, contained on or available through this website, is for general information purposes only. Please contact your physician to form a plan that addresses you or your child's specific needs" wherever space is allotted."

11) We will make regular presentations about the research to community organizations including neighborhood groups, parent groups, and church groups.

12) Participants will be recruited through the Research Experience Participation (REP) program in the Psychology Department at the University of Minnesota. Study information will be available on the REP website. Research staff may introduce the study in relevant UMN courses and emails with study information may be distributed in relevant UMN courses.
13) Descriptions of the study will also be posted on research study websites, like ClinicalTrials.gov and StudyFinder.
14) We will be recruiting for the study at the Minnesota State Fair Driven to Discover Research Facility (D2D) at a recruitment booth for our research lab. We will have flyers and brochures about the study at the booth. For parents or guardians who tell us they have a daughter in our study age range (16-18), or the individual themselves (18 to 24) we will encourage them to complete a permission to contact form to indicate their interest in being contacted about the study and gather their contact information using a secure REDCap form. We will be giving away small prizes for those who sign their daughter up to be contacted.

Sites for Recruitment:

1) UMN Psychiatry Clinic: Potential participants will be contacted from the clinic registry. In addition, a flyer will be posted outside of this clinic where potential participants will self-identify and potentially contact research staff to inquire more information.
2) Fairview/M Health: Fairview Health Services will identify potential participants from a clinical data repository then mail an IRB-approved study recruitment letter to their patients on behalf of the research team.
3) Community bulletin boards: Flyers will be posted on community boards such as in coffee shops, cafes and community centers, where permission is granted.
4) Internet advertisement: Similar advertisements will be posted through internet forums where IRB approved. We will engage BUMP marketing with IRB approved materials for internet advertisement.
5) High schools: We will engage local high schools to gauge interest in presentations to students or community groups about health topics related to brain development, adolescent health, self-harm and mental health. We will

also reach out to high school counselors to build awareness and encourage referrals for the research study.

6) Clinical groups: We will engage local clinical groups that may see the target population as patients to build awareness and encourage referrals for the research study.
7) Community groups: We will engage community groups
8) Minnesota State Fair Driven to Discover Research Building: We will have a booth at this building to recruit participants for the research study.

The following recruitment materials are in production and will be used for recruitment. Once finished, copies will be provided to Advarra IRB for approval.

1) Flyer (for public display outside outpatient clinic, potential participant self-identify)
2) Flyer (for public display outside inpatient clinic, potential participant self-identify)
3) Flyer (for public display in community, potential participant self-identify)
4) Flyer (for display to clinicians/clinical groups, potential participants identified by clinician)
5) Internet advertisement (for public display, potential participant self-identify)
6) Letter to Fairview patient (mailed directly to potential participant, identified by Fairview staff via repository)
7) Study summary shown to potential participants when accessing online screening form)
8) REDCap online screen (automated screening form for potential participant eligibility)

Screening

Screening documentation: A screening log will be kept to record all candidates, reasons for ineligibility or reason for electing to not participate.

Consent

We anticipate to consent up to 80 people. The consent process with study candidates and/or their parent/guardian will be conducted by the research coordinator or principal investigator and will take place at the very first scheduled visit, Day 0, before the clinical assessment. They will receive copies of the parental consent, assent (if applicable), email authorization and HIPAA forms via encrypted email or USPS

mail at least 24 hours before this appointment. The initial consent process will take place with research staff in a previously reserved room within the Ambulatory Research Center (ARC) at the University of Minnesota Department of Psychiatry (F212) Fairview Riverside West Building, or at the Research in Adolescent Depression Lab at the University of Minnesota Medical School, or via video conference. During the initial consent visit staff will review the consent forms highlighting the most salient points such as study title, purpose, procedures, risks, benefits, alternatives, confidentiality, research related injury, voluntariness, and any additional signing/initial blocks such as permission to re-contact in the future and share data outside of this research study. Talk back method will be used during the consent visit to ensure study candidate and/or their parent/guardian (if applicable) are understanding these points. In addition, an approved version of the UCSD Brief Assessment of Capacity to Consent specific to this study will be used with all candidates during the consent process. Candidates that are not able to complete the UBACC or score less than 2 points on each question will not be consented and enrolled into the study. If a potential participant scores 0 or 1 on any question the information pertinent to that question will be reviewed and discussed with the participant. If the potential participant is then able to answer the question in full and research staff determines the potential participant fully understands the material, they will be scored the 2 points and then consented and enrolled. In addition to University and department mandatory consent training for appropriate research staff, all new staff will be trained in consent for this research study including observing the consent process, mock consent and observed for consent process. Throughout the course of the research study, participants will be gauged of their comprehension of scheduled procedures as well as comfort and willingness to complete these procedures by staff at each visit/procedure. All participants participating in this study or their LARs will be required to provide prospective informed consent. This will be completed via remote consent procedures (including e-consent with electronic signatures) which complies with requirements outlined in Title 21 CFR Part 11. We will email the ICF to the participant or LAR via a REDCap link. We will take special precautions to protect confidentiality (e.g. verbally (over video call) provide the participant and/or LAR with a unique code generated by REDCap that will grant access to ICF). The person consenting the participant or LAR will have the same consent discussion via phone or zoom that they would have had in-person (including asking questions to gauge comprehension and answering the participant's or LAR's questions). If the participant or LAR consents, they will complete and electronically sign the ICF (in all appropriate sections) directly in REDCap. Once the ICF (signed & dated by the participant or LAR) is received by the research team, the study team member who explained the study will electronically sign the appropriate signature line with the current date. They will then email a copy of the fully executed ICF back to the participant. They will document the consent process as appropriate. Study procedures will not begin until all aspects of the remote consent process is complete. A copy of the ICF will not be included in the participant's Electronic Health Record.

For those age 16 or 17, parent/guardian consent will be obtained from at least one parent. Parent/guardian consent will be attempted to be obtained from both parents unless one parent is deceased, unknown, incompetent or not reasonably available due to travel or work. Permission will be obtained from someone other than a parent only when that person is a legal guardian of the potential participant. Assent will be obtained and documented from all participants that meet criteria and are willing to consent that are age 16 or 17. Documentation will be obtained by child signing Assent Form (ink or electronic signature if conducted via video conference), along with research staff personnel obtaining consent (ink or electronic signature if conducted via video conference).

Randomization and Double-Blind: Participants that meet criteria for entry into the study based on the online screen and the clinical interview will be randomized to active NAC (low dose versus high dose), versus PBO. We will implement an adaptive randomization procedure similar to stratification called minimization. The purpose of the procedure is to ensure that the 3 groups are similar with respect to key demographic and clinical variables such as age, depression severity, NSSI severity and medication use. These variables will be shared by the research coordinator in a de-identified file with the study statistician, who will use the information to complete the randomization procedure. She will then share the group assignment with IDS. All participants, family members, and the entire research team will be blinded to active NAC versus PBO assignment.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Study intervention: N-acetylcysteine (NAC); 600mg capsules

NAC is a natural product that may have promise for treating NSSI in adolescents. NAC is the N-acetyl derivative of the amino acid L-cysteine and a precursor of the antioxidant glutathione. It can be found in health food stores with other nutritional and dietary supplements. The bioavailability and pharmacokinetic (PK) properties of intravenous (IV) and oral NAC have already been established in humans (Holdiness 1991).

Dispensing and administration of intervention: Participants will be provided with a 4-week supply of study drug, plus an additional four days, on Day 1 (Visit 2) by the research coordinator. The study drug is a gelatin 0-size capsule, with a dose of 600mg per capsule. Participants will be provided with an extra four-day supply of study drug in case scheduling challenges occur, in order to ensure adequate dosing of study drug at post-dose biological sampling (PK analysis and 7T MRI scan). The research team will request subjects use a paper medication diary (calendar) or complete a survey via REDCap to optimize and track medication adherence. The participant will have the option of which method they would prefer to use (paper or survey). Participants will indicate this preference through the Medication Tracking Preference Form via

REDCap. If they prefer to receive the surveys, the participants will be asked whether they preferred survey delivery via text or email. The online survey will ask the participants to indicate whether they had taken their daily medication in the morning and afternoon. The participant will not be required to use either of the tracking methods, paper or survey. The research coordinator will contact the participants on day 3, 4 or 5 of study participation to check in about their adherence, their chosen method of adherence tracking and experiences with the study drug. They will be instructed to take 5 identical-appearing capsules in the morning and 4 in the evening (5400mg/day, n=12; 3600mg/day, n=12; PBO, n=12; participants in high-dose group will be given 5 active capsules in the morning and 4 active capsules in the evening, participants in low-dose group will be given a mix of active and placebo capsules (3 active capsules and 2 placebo capsules in the morning, and 3 active capsules and 1 placebo capsule in the evening), and the placebo group will receive all placebo capsules. Capsules will be taken by mouth, beginning Day 1(Visit 2) until the final visit (Visit 4; Day 28). Study capsules will be packaged to ensure that the correct capsules are taken at the correct day and time.

NAC side effect reports in published studies of oral NAC have ranged from 81% reporting no side effect (N=1,392)(Tattersall, Bridgman, and Huitson 1983) to no significant between group differences in adverse events (N=75)(Berk et al. 2008). All potential adverse effects from NAC would be acute and reversible.

Oral NAC may cause gastrointestinal adverse effects including nausea, stomach discomfort, diarrhea, and constipation, as well as headache and chest tightness (Behr et al. 1997; Oldemeyer et al. 2003). Adverse effects published resolved with discontinuing NAC demonstrating that effects would be acute and reversible.

For participants that experience adverse effects likely or probably related to study drug the participant will be instructed to diminish their dose by 1 capsule daily until the absence of the adverse effect occurs (i.e. from 9 to 8 to 7, etc., until adverse effect is absent). The goal of tapering down to tolerance will be to allow the participant to remain in the study and tolerate the study drug. If a taper is needed, IDS will re-package the study drug so that the blind is maintained and the correct number of active pills are taken.

5.2 Handling of Study Interventions

The SW854 product will be procured from the company Swanson Health Products. It will then be given to the Investigational Drug Service (IDS) Pharmacy. IDS will manufacture matching placebo. The placebo capsules are identical in appearance and smell and taste as the active capsules. UMN IDS will handle all NAC and PBO allocation and dispensing. All participants, family members, and the entire research team will be blinded to active NAC versus PBO assignment. If there are any leftover capsules not taken by a participant, these will be returned to IDS, recorded and disposed of according to University of Minnesota IDS procedures.

5.3 Concomitant Interventions

5.3.1 Allowed Interventions

Participants will be allowed to continue their previous psychiatric medications and psychotherapy. A requirement for study entry is having psychiatric medications dose-stable for one month. Changes to psychiatric medications during the study are strongly discouraged, and we will ask participants to promptly notify us of any changes. If such changes occur, the Principal Investigator and co-investigators Coles, Kartha or Cloyd will review the relevant information and potential interactions. If it is determined that the new medication(s) raise potential safety concerns while being taken with NAC, the participant will be advised to stop taking study drug and still complete study visits as planned. Otherwise, the participant will continue. These changes will be documented and incorporated into sensitivity analyses as described in section 9.6.

5.3.2 Required Interventions

N/A

5.3.3 Prohibited Interventions

Participants may not have previously taken NAC or glutathione on a regular basis for 6 months prior to, or during, enrollment in the study. All psychotropic medications must be dose stable for 1 month prior to, and during, enrollment.

5.4 Adherence Assessment

Adherence will be defined by completion of all in-person study visits (Visits 1, 2, 3, and 4), medication adherence of at least 80% (based on medication tracking and pill count at Days 14 and 28) and completion of at least 80% of in-person and at-home measures (complete at least 28/35 requested measures).

6. STUDY PROCEDURES

6.1 Schedule of Activities

Measure/Activity/Event	Visit 1 (Day 0)	Visit 2 (Day 1)	Day 3, 4, or 5	Day 7	Visit 3 (Day 14)	Day 21	Visit 4 (Day 28)	24-72 hours after final dose
Informed Consent Form (HIPAA/Consent/Assent)	X							
Unsecured Email Authorization Form	X							
Guidelines And Consent For Text Message Correspondence Form	X							
Mini International Neuropsychiatric Interview (MINI)	X							
Wechsler Abbreviated Scale for Intelligence-II (WASI-II)	X						X	
Edinburgh Handedness Inventory	X							
Demographics Form	X							
Childhood Trauma Questionnaire	X							
Patient Health Questionnaire (PHQ-9)		X		X	X	X	X	
Ongoing Medication Use and Changes: Initial Visit		X						
Ongoing Medication Use and Changes: Subsequent Visit				X	X	X	X	
Antidepressant Medications	X							
Any Psychotropic Medications	X							
Treatment History	X							
Alexian Brothers Urge to Self-Injure (ABUSI)		X					X	
Beck Depression Inventory (BDI-II)	X						X	
Self-Injurious Thoughts and Behaviors Interview (SITBI)	X							
Beck Scale for Suicidal Ideation (BSS)		X					X	
Inventory of Statements About Self-Injury-Lifetime (ISAS-Lifetime)	X							
Inventory of Statements About Self-Injury-Since Last Visit (ISAS-SLV)		X		X	X	X	X	
Deliberate Self-Harm Questionnaire, Part III Mood (DSHQ-M)		X					X	
Cash Choice Task		X					X	
Medication Side Effect Checklist		X		X	X	X	X	
Distress Tolerance Scale (DTS)	X							

Measure/Activity/Event	Visit 1 (Day 0)	Visit 2 (Day 1)	Day 3, 4, or 5	Day 7	Visit 3 (Day 14)	Day 21	Visit 4 (Day 28)	24-72 hours after final dose
Medication Tracking Preference Form		X						
Provided with Medication Diary (if hard copy is elected)		X			X			
MRI Safety Screen		X					X	
Urine Toxicology Screen		X					X	
Pregnancy Test		X					X	
MRI		X					X	
Blood Sample (PK and biomarkers)		X						
Provided with 32 day supply of intervention (NAC/PBO)		X						
Final dose of intervention							X	
IV catheter/ needle stick(s) and blood samples (x 3-5) (PK)							X	
Phone call (assess adverse effects, questions, concerns)			X					X
Pill count					X		X	

6.2 Description of Evaluations

6.2.1 Screening Evaluation

Consenting Procedure

Consent procedures: The consent process with study candidates and/or their parent/guardian will be conducted by the research coordinator or principal investigator and will take place at the very first scheduled visit (in person or through video conferencing), Day 0, before the clinical assessment. They will receive copies of the parental consent, assent (if applicable), email authorization and HIPAA forms via encrypted email or USPS mail at least 24 hours before this appointment. The initial consent process will take place with research staff in a previously reserved room within the Ambulatory Research Center (ARC) at the University of Minnesota Department of Psychiatry (F212) Fairview Riverside West Building, or the Research in Adolescent Depression Lab at the University of Minnesota Medical School, or via video conference. During the initial consent visit staff will review the consent forms highlighting the most salient points such as study title, purpose, procedures, risks, benefits, alternatives, confidentiality, research related injury, voluntariness, and any additional signing/initial blocks such as permission to re-contact in the future and share data outside of this research study. Talk back method will be used during the consent visit to ensure study candidate and/or their parent/guardian (if applicable) are understanding these points. In addition, an approved version of the UCSD Brief Assessment of Capacity to Consent specific to this study will be used with all candidates during the consent process. Candidates that are not able to complete the UBACC or score less than 16 total points will not be consented and enrolled into the study. If a potential participant scores 0 or 1 on any question the information pertinent to that question will be reviewed and discussed with the participant. If the potential participant is then able to answer the question in full and research staff determines the potential participant fully understands the material, they will be scored the 2 points and then consented and enrolled. In addition to University and department mandatory consent training for appropriate research staff, all new staff will be trained in consent for this research study including observing the consent process, mock consent and observed for consent process. Throughout the course of the research study, participants will be gauged of their comprehension of scheduled procedures as well as comfort and willingness to complete these procedures by staff at each visit/procedure. All participants participating in this study or their LARs will be required to provide prospective informed consent. This will be completed via remote consent procedures (including e-consent with electronic signatures) which complies with requirements outlined in Title 21 CFR Part 11. We will email the ICF to the participant or LAR via a REDCap link. We will take special precautions to protect confidentiality (e.g. verify with the participant that the email is correct and it is acceptable to send the consent in this way). The person consenting the participant or LAR will have the same consent discussion via phone or zoom that they would have had in-person (including asking questions to gauge comprehension and answering the participant's or LAR's questions). If the participant or LAR consents, they will complete and electronically sign the ICF (in all appropriate sections) directly in REDCap. Once the ICF (signed &

dated by the participant or LAR) is received by the research team, the study team member who explained the study will electronically sign the appropriate signature line with the current date. They will then email a copy of the fully executed ICF back to the participant. They will document the consent process as appropriate. Study procedures will not begin until all aspects of the remote consent process is complete. A copy of the ICF will not be included in the participant's Electronic Health Record.

For those age 16 or 17, parent/guardian consent will be obtained from at least one parent. Parent/guardian consent will be attempted to be obtained from both parents unless one parent is deceased, unknown, incompetent or not reasonably available due to travel or work. Permission will be obtained from someone other than a parent only when that person is a legal guardian of the potential participant. Assent will be obtained and documented from all participants that meet criteria and are willing to consent that are age 16 or 17. Documentation will be obtained by child signing Assent Form *(ink or electronic signature if conducted via video conference)*, along with research staff personnel obtaining consent as described above.

Participants will be able to opt into receiving unencrypted email communication and texts from study staff for reminders about appointments or instructions. This process is discussed in Section 11.2 Informed Consent Forms.

Screening

Initial screening: Potential participants who respond to advertisements will be directed to an online, IRB approved study summary and will be prompted to complete a REDCap self-report survey to assess initial eligibility. For those who meet eligibility criteria and express interest in participating in the study, we will schedule candidates for an in-person or video conference meeting to fully explain study procedures and risks (i.e. Visit 1). At Visit 1, assessment interviews with participants will be conducted directly after the consent process and will finalize eligibility. Information obtained from online screen (age, gender, health history, NSSI frequency, diagnoses, medication history) will be confirmed at this time.

Assessment of General Psychopathology and Demographic Information: We will screen for the presence of DSM-5 psychiatric diagnoses (American Psychiatric Association 2013b) at Visit 1 using the Mini International Neuropsychiatric Interview (MINI; (D. V. Sheehan et al. 1997). Clinicians formulate the diagnosis combining all clinical information. SITBI, Demographics Form, Anti-Depressant Medications form, Any Psychotropic Medications form, and Treatment History form will all be completed at Visit 1. MRI Safety Screen will be completed at Visit 2 prior to neuroimaging procedures.

All screening evaluations must be completed in 60 days from screen to Visit 2 (Day 1) and in 30 days from Visit 1 (Day 0) to Visit 2 (Day 1).

For the medication history criterion (dose stable for 1 month) the timeline will measure from date of consent (i.e. psychotropic medications dose stable 1 month prior to consent date).

An eligibility checklist will be used to fully document all pieces of eligibility. The following evaluation measures/forms will be used in screening eligibility:

- Online Screen Form
- Mini International Neuropsychiatric Interview (MINI)
- Self-Injurious Thoughts and Behaviors Interview (SITBI)
- Antidepressant Medications
- Any Psychotropic Medications
- Treatment History Form
- MRI Safety Screen
- Inventory of Statements About Self-Injury-Lifetime (ISAS-Lifetime)
- Eligibility Checklist

The following table outlines each visit and measure used to assess each eligibility criterion:

Table 2 – Screening Measures and Visits

Criterion Assessed	Inclusion/Exclusion	Visit/Event	Measure/Form/Survey
16-24 years old	Inclusion	Online screening	Online Screen
Female	Inclusion	Online screening	Online Screen
Generally medically healthy	Inclusion	Online screening	Online Screen
At least 1 NSSI episode in past 2 months	Inclusion	Online screening	Online Screen
		Visit 1	SITBI
			ISAS-Lifetime
≥ 5 past episodes of NSSI with significant tissue damage (e.g. skin is broken or bruised, and/or scars resulted from the injury)	Inclusion	Online screening	Online Screen
		Visit 1	SITBI
			ISAS-Lifetime
Psychotropic medications are dose-stable for 1 month.	Inclusion	Online screening	Online Screen
		Visit 1	Antidepressant Medications
			Any Psychotropic Medications
Any MRI contraindications (e.g. metal plates, claustrophobia, braces, implanted devices)	Exclusion	Online screening	Online Screen
		Visit 2	MRI Safety Screen
		Visit 4	
Male	Exclusion	Online screening	Online Screen
Serious medical illness that involves the central nervous system, or which requires treatment that significantly impacts the central nervous system.	Exclusion	Online screening	Online Screen
Current Substance Use Disorder (except mild cannabis disorder, mild alcohol use disorder, or alcohol or substance use disorder in early remission with at least 3	Exclusion	Online screening	Online Screen
		Visit 1	MINI

months abstinence)			
Primary psychotic disorder (e.g. schizophrenia, schizoaffective disorder, schizophreniform disorder)	Exclusion	Online screening	Online Screen
		Visit 1	MINI
Neurodevelopmental disorder such as mental retardation or autism	Exclusion	Online screening	Online Screen
		Visit 1	MINI
			Treatment History Form
Changes in psychotropic medications in past 1 month	Exclusion	Online screening	Online Screen
		Visit 1	Antidepressant Medications
			Any Psychotropic Medications
Taken NAC or glutathione on a regular basis in the past 6 months	Exclusion	Online screening	Online screen
		Visit 1	Treatment History
Currently pregnant, or planning to become pregnant	Exclusion	Online screening	Online screen
		Visit 2	Pregnancy Test
		Visit 4	
Allergy/sensitivity to N-acetylcysteine.	Exclusion	Online screening	Online screen
		Visit 2	
Inability or unwillingness of individual or legal guardian/representative to give written informed consent.	Exclusion	Visit 1	Consent forms

6.2.2 Enrollment, Baseline, and/or Randomization

Enrollment

Enrollment will be defined as the time at which the participant is randomized. Consent will occur at Visit 1 (Day 0). Randomization will occur after Visit 1 (Day 0), when it is confirmed that all eligibility criteria are met, and before Visit 2 (Day 1). There will be a maximum 30-day window between Visit 0 and Visit 1. Enrollment date will be recorded in OnCore (subject specific calendar), and Enrollment Log.

Baseline Assessments

Visit 1 (Day 0) Initial Assessments:

- Mini International Neuropsychiatric Interview (MINI)
- Self-Injurious Thoughts and Behaviors Interview (SITBI)
- Edinburgh Handedness Inventory
- Demographics Form
- Anti-Depressant Medication Form
- Any Psychotropic Medication Form
- Childhood Trauma Questionnaire (CTQ)
- Beck Depression Inventory-II (BDI-II)
- Inventory of Statements About Self-Injury (ISAS-Lifetime)
- Distress Tolerance Scale (DTS)

Visit 2 (Day 1) Baseline Assessments/Activities:

- Beck Scale for Suicidal Ideation (BSS)
- Patient Health Questionnaire-9 (PHQ-9)
- Alexian Brothers Urge to Self-Injure (ABUSI)
- Deliberate Self-Harm Questionnaire, Part III Mood (DSHQ-M)
- Cash Choice Task
- Side Effect Checklist
- Ongoing Medication Uses and Changes: Initial Visit
- MRI safety screen
- Blood draw
- Urine toxicology screen
- Pregnancy test
- Neuroimaging procedure (MRI brain scan)

- Wechsler Abbreviated Scale for Intelligence-II (WASI-II)

Intervention dispensed with instructions (32-day supply)

Clinical Assessment-Assessment of General Psychopathology and Demographic Information: To describe our sample and to determine criteria for ineligibility, we will screen for the presence of Diagnostic and Statistical Manual of Mental Disorders-5th edition (DSM-5) (American Psychiatric Association 2013a) psychiatric diagnoses (American Psychiatric Association 2013b). We will conduct a clinical interview with the participant using the Mini International Neuropsychiatric Interview (MINI; (D. V. Sheehan et al. 1997) for all participants. Assessment interviews will be conducted by trained research team members, directly after the consent process. Consent and baseline clinical assessment are expected to take 2 to 3 hours to complete. The Wechsler Abbreviated Scale for Intelligence-II (IQ; (Wechsler 2011) will be used to estimate intellectual functioning, to be completed on Day 1 or 28. The Edinburgh Handedness Inventory (Oldfield 1971) will be used to determine handedness, which will be incorporated into the analysis. We will monitor the groups' equality with respect to socioeconomic status using the Hollingshead Four Factor Index of Social Status (Hollingshead 1975) after collecting demographic information with a self-report form. We will also collect past antidepressant medication trials with an Anti-Depressant Medication form, other psychotropic medications with Any Psychotropic Medications form and treatment history with Treatment History form, all via REDCap. The Child Trauma Questionnaire (CTQ) (Bernstein D.P & Fink 1998) includes 6 questions about past trauma and 7 about recent trauma. Depression severity will be measured using the Beck Depression Inventory-II (BDI-II; (Osman et al. 2004) and the Patient Health Questionnaire (PHQ-9) (Kroenke et al. 2001). We will also administer the medication side effects checklist to assess baseline symptom overlap with responses to this checklist at later stages of the study. Assessment of NSSI and Suicidality. We will use the Self-Injurious Thoughts and Behaviors Interview (SITBI) (Nock et al. 2007) to elicit NSSI history (age of onset, total past episodes, episode frequency, reasons for NSSI, thoughts about NSSI, and other information) and use the Inventory of Statements about Self-Injury (ISAS-Lifetime) to assess the functions and frequency of behaviors of NSSI (Klonsky & Glenn 2009). We will use the Alexian Brothers Urge to Self-Injure (ABUSI) scale to assess urges to engage in NSSI. We will use the Beck Scale for Suicidal Ideation (BSS; (Beck, Kovacs, and Weissman 1979) to assess current suicidal ideation, including active suicidal desire, specific suicide plans, and passive suicidal desire. To retrospectively assess mood before, during and after NSSI events we will use the Deliberate Self Harm Questionnaire, Part III Mood (DSHQ-M). We will use the Distress Tolerance Scale (DTS) to assess emotional distress tolerance (Simons & Gaher 2005). We will use the one-question Cash Choice Task as a delay discounting measure to further assess executive functioning (Wulfert et al., 2002). Finally we will use the Side Effect Checklist to assess baseline symptoms on this form at Day 0, before participant takes study drug/placebo. The demographic history form, antidepressant medication form, any psychotropic medications form, treatment history form, PHQ-9, ISAS-SLV, ISAS-Lifetime, ABUSI, DTS, SITBI, Cash Choice Task, Side Effect Checklist, and

Ongoing Medication Uses and Changes: Initial Visit will be available online via REDCap. Paper versions will be available if necessary in the event that REDCap is down, computer unavailable, etc. Participants who are unable to complete all online self-report questionnaires in-person due to time constraints will be emailed an access code for each questionnaire and a link to the REDCap website. Upon opening the website link, they will be prompted to enter their access code in order to fill out questionnaires. Following the clinical evaluation, the study team will review all clinical information and determine final eligibility to decide whether the participant will move forward to randomization. A summary of the diagnostic findings will be provided to the participant (and for those aged 16-17 years, the parent). This clinical information will be given verbally, and by request we will additionally provide a written summary of the clinical findings. All potential participants that inquire about study participation but do not meet criteria for participation will be offered referral guidance for clinical care to Fairview and University of Minnesota Psychiatry services in cases where care is not currently established.

Biological Assessments: Following the informed consent, baseline clinical assessment and (if eligible) randomization, participants will be invited to the CMRR for a 2-3 hour visit. During the global COVID pandemic, for all in-person visits, upon arrival, participants will complete COVID screen, follow pandemic related safety precautions including use of face mask and social distancing as outlined in the approved sunrise plan submitted by PI Dr. Cullen. They will then complete a blood draw, a urine toxicology screen, pregnancy test and an MRI safety screen, followed by neuroimaging procedures as described below. For the urine tests, the participant will provide a urine sample, and the research staff will apply the urine pregnancy test strip and the urine toxicology test strip according to the package instructions. Research staff will record the results in the CRF and discard the urine sample and the test strips according to standard CMRR procedures. If urine pregnancy test is positive, staff will not complete the MRI scan on the participant. The participant will also no longer meet criteria for the study, and will be removed from the study. If the toxicology screen is positive, results will be noted on the MRI CRF form and the participant will continue on with the MRI scan. Participants that do not pass the MRI safety screen will not receive MRI scan and will be allowed to remain in the study. Parents of participants, even those under 18 years of age, will not be informed of pregnancy or urine toxicology test results. The research team will utilize the CMRR Center's Subject Information Form and adhere to the SOP during enrollment of all research participants in this protocol. The CMRR Center's Subject Information Form and SOP are IRB approved under the CMRR Center Grant (HSC# 1406M51205) and information regarding these procedures is publicly available on the CMRR website (CMRR Policies / Procedures).

Randomization

Randomization and Double-Blind: Participants who meet criteria for entry into the study (based on the online screen and the clinical interview) and consent to participate will be randomized to active NAC high dose versus active NAC low dose versus PBO (1:1:1). We will implement an adaptive randomization procedure called minimization. The purpose of the procedure is to ensure that the 3 treatment groups

are similar with respect age, NSSI severity, and medication use. Specifically, the minimization procedure will balance age groups <19 vs. 19+ years, NSSI severity category mild vs. moderate vs. severe; and anti-depressant use vs. no anti-depressant use. Following the evaluation, the study coordinator will share numerical values for these variables along with the subject ID number through a secure system (Box Secure Storage) with the study statistician, Dr. Eberly. Dr. Eberly will use participant information to assign a blinded randomization group (A, B, or C) using the minimization procedure.

The study statistician will then pass the blinded group assignment to IDS. Before the first participant is randomized, IDS will randomly assign "NAC high" "NAC low" and "PBO" to the letters A, B, and C; this assignment (of treatment name to treatment letter) will not be communicated to anyone outside of IDS until the study is complete and the study team is ready to break the blind. Randomization will occur after Visit 1 (Day 0), when it is confirmed all eligibility criteria are met and before Visit 2 (Day 1), with a maximum 30 day window between them.

6.2.3 Blinding

All participants, family members, and study staff (including the study statistician) will be blinded to treatment assignment. Study staff will not discuss data collected with the research participant. The research coordinator will be the main person communicating with the participant and completing in-person visits with them; this staff member will not engage in any data analysis while study is actively enrolling and conducting visits. The IDS pharmacist will be authorized to break the blind in the event of a medical emergency in which the medical team caring for the participant feels that knowledge of the identity of the study drug is necessary. Participants will have IDS emergency contact information. In the event breaking the blind is necessary, IDS pharmacist will communicate to the participant and their medical care team information about the study intervention (NAC versus placebo, dose, inactive ingredients, etc.).

Any interim reports on the data generated by the research team for the Independent Monitoring Committee (IMC) will be generated as the IMC requests. If this occurs we will bring onto the project a statistician from the Biostatistical Design and Analysis Center (BDAC) within the University of Minnesota's CTSI to handle the unblended analyses. The breaking of the treatment blind for all participants will not occur until the last consented participant has completed their treatment period or earlier if the study is stopped early based on IMC recommendation.

6.2.4 Follow-up Visits

Day 7 (+/- 2 days) Assessments/Activities:

- At-home online assessments:
    - PHQ-9
    - Inventory of Statements About Self-Injury, since last visit (ISAS-SLV)
    - Side Effect Checklist

- Ongoing Medication Uses and Changes: Subsequent Visits

Visit 3 (Day 14 +/- 2 days) Assessments/Activities:

- In-person or video conference assessments:
  - PHQ-9
  - ISAS-SLV
  - Side Effect Checklist
  - Ongoing Medication Uses and Changes: Subsequent Visits
- Capsules count previous dispensed amount, check medication tracking table or REDCap survey records (participant counts pills over zoom during 14-day visit if it is conducted virtually)

Day 21 (+/- 2 days) Assessments/Activities:

- At-home online assessments:
  - PHQ-9
  - Inventory of Statements About Self-Injury, since last visit (ISAS-SLV)
  - Side Effect Checklist
  - Ongoing Medication Uses and Changes: Subsequent Visits

6.2.5 Completion/Final Evaluation

In the context of the global pandemic, we have shortened the final visit to reduce the time that participants will be required to be indoors in the CMRR. Thus, during the pandemic, we will utilize an abbreviated protocol (3 hours instead of 8, with fewer blood draws.) The PI will determine if and when to resume the original PK sample collection scheme (longer protocol) for visit 4 once it is safe to do so based on the status of the global pandemic and the guidance from the University.

Visit 4 (Day 28-32 days) Assessment/Activities per abbreviated PK sample collection scheme:

- COVID screen
- MRI Safety Screen
- Urine Toxicology Screen
- Pregnancy Test
- IV catheter placement or a series of needle stick(s) if the participant prefers
- 3-5 blood collections
- Neuroimaging procedure (MRI brain scan)
- PHQ-9

- BDI-II
- BSS
- ABUSI
- ISAS-SLV
- DSHQ-M
- Cash Choice Task
- Side Effect Checklist
- Ongoing Medication Uses and Changes: Subsequent Visits

Participants will return to the CMRR for a 3-8 hour visit ~4-weeks after beginning study drug, on Day 28 - 32 and present their remaining medications for capsule count to assess drug compliance. This visit will entail a repeat of the baseline neuroimaging procedure and multiple blood collections for PK analysis. The participant will be asked if they would prefer placement of IV catheter versus a series of needle sticks every time a blood sample is collected. We will collect an initial blood sample to measure trough NAC and CYS levels, as well as oxidative stress measurements detailed below. Then, participants will take their final oral dose. We will collect additional blood samples (2-5) starting at 15 minutes and ending at ~120-300 minutes following the final oral dose to measure NAC and CYS levels (abbreviated COVID-19 protocol: just 2 post-NAC dose blood draws, one right before and one right after the MRI scan). Neuroimaging will begin ~15 minutes after the final dose and will last ~60-90 minutes. Following this MRI scan, participants will be interviewed to ascertain medication side effects using a checklist. Although this study is not designed to examine clinical effects, to allow for exploratory analyses examining links between concurrent symptoms and biology, we will ask participants to complete the ABUSI, an abbreviated form of the SITBI (modified to query the NSSI thoughts, urges and behaviors since the prior clinical assessment), the ISAS-SLV to assess NSSI episodes, BDI-II, PHQ-9 and BSSI to assess depression symptoms and current suicidal thoughts and behaviors, the DSHQ-M to assess mood in relationship to NSSI episodes, and the one-question Cash Choice Task as a delay discounting measure (Wulfert et al., 2002) . The PHQ-9, ISAS-SLV, ABUSI, DTS, Cash Choice Task, Side Effect Checklist, and Ongoing Medication Uses and Changes: Subsequent Visits will be available online via REDCap. Paper versions will be available if necessary (REDCap down, computer unavailable, etc.) Participants who are unable to complete all online self-report questionnaires in-person due to time constraints will be emailed an access code for each questionnaire and a link to the REDCap website. Upon opening the website link, they will be prompted to enter their access code in order to fill out questionnaires. Upon administration of the final dose of study drug, participants will be instructed to inform study staff of any potential adverse effects that occur after leaving the research facility. In addition, 24-72 hours after the final dose of NAC, participants will receive a phone call from study staff to assess any potential adverse effects after the final dose of study drug and address any lingering questions/concerns about study participation and/or referral suggestions for future

clinical care addressing NSSI or psychiatric symptoms/co-morbidities. If the participant unexpectedly needs to have their final visit rescheduled, we will prescribe an additional small supply of study medication to allow the participant to continue taking study medication up until the final visit, to a maximum of 35 days.

Payment

Participants will be compensated directly using the Greenphire ClinCard. Each task completed will have attached a specific compensation amount. Once the task is completed research staff will load the amount attached to that task on to the participants Greenphire ClinCard. In cases where a participant only completed a portion of a visit, a portion of the compensation would be provided depending on what was completed. If any visit or part of the visit needs to be repeated for reasons beyond participants' control, they would be compensated.

1) Day 0 (in-person or through video conferencing) (visit 1): Initial clinical assessment: $40
2) Day 1 (in-person visit 2): Baseline assessments, MRI scan and blood draw: $50
3) Day 7 (at home): Online questionnaires: $10
4) Day 14 (in-person or through video conferencing) (visit 3): Mid-study visit questionnaires: $10
5) Day 21 (at home): Online questionnaires: $10
6) Day 28 - 32 (in-person visit 4): Post-dose MRI and blood samples (over 3-4hours per abbreviated PK sample collection plan or 6-8 hrs): $200
7) Total Compensation Per Participant for R61 = $320. Participants may also choose to receive course extra credit as partial or total payment instead of cash (Research Experience Points) for their participation in this study (each ½ hour of assessment is worth 1 REP point).

Research Related Injury

There is no compensation for research related injury. The participant or the participants insurance company will be financially responsible for any injury occurring because of participating in this study.

7. SAFETY ASSESSMENTS

Safety will be monitored throughout the study. Here we review the risks associated with the study and how we will monitor for these risks.

1. Monitoring for safety related to taking NAC: Side effect reports in published studies of oral NAC have ranged from 81% reporting no side effects (N=1,392)(Tattersall, Bridgman, and Huitson 1983) to no significant between group differences in adverse events (N=75)(Berk et al. 2008). Oral NAC may cause gastrointestinal adverse effects including nausea, stomach discomfort,

diarrhea, and constipation, as well as headache, chest tightness, rash and/or itching. (Behr et al. 1997; Oldemeyer et al. 2003). These problems were reported as resolved following discontinuation of NAC. Adverse effects due to NAC will be monitored using the Side Effects Checklist. Participants who experience adverse effects which are bothersome and which are deemed likely or probably related to study drug will be instructed to decrease their dose by 1 capsule daily until they tolerate the study drug.

2. Monitoring for safety related to IV catheter placement and blood collection: The risks of the IV catheter and blood collection include pain at the site, bleeding, bruising, fainting and/or dizziness, superficial phlebitis and infection. These risks will be lessened by using trained technicians to perform this procedure. These potential adverse effects would be acute and reversible.
3. Monitoring for safety related to suicide risk: Our study population is at increased risk for suicide attempts (Horwitz, Czyz, and King 2015; Tang et al. 2011; Victor and Klonsky 2014). Suicide risk will be monitored throughout the study in the form of questionnaires and interviews. If a concern for imminent suicide risk is identified, the study team will conduct a more in-depth safety assessment and will engage the adolescent in safety planning. As indicated, this safety assessment and planning may include the parent(s), other family members, treatment team, and / or referral for emergency services / hospitalization. Refer to Supplement IV: RAD Lab procedure on suicide and self-harm behavior risk.
4. Monitoring for safety related to clinical assessment: Some of the questions that participants will be asked may make them feel uncomfortable or upset. These pertain to psychiatric and medical history, behavioral and psychiatric symptomatology. Participants are informed that they do not have to answer any question that makes them feel uncomfortable. Clinicians interviewing the participants will remind them of this, as well as use empathy to gauge their discomfort. Staff will be sufficiently trained to handle these situations. If participants are not able to share enough critical information needed for the study (e.g. inclusion and exclusion criteria, NSSI etc.) the participant will be withdrawn.
5. Monitoring for safety related to MRI scanning: The MRI scanning device will be a 7.0 Tesla MRI scanner. This device has been evaluated by the FDA as having non-significant risk for persons more than one month of age and data already reviewed by the UMN IRB indicate that the 7.0 Tesla magnetic field does not pose a significant risk to human volunteers. All participants will be screened according to CMRR policies to minimize risks to participants. If participants are not able to tolerate the scanning session, it will stop and they will exit the study. These are the specific risks related to MRI scanning:
    a) Exposure to high magnetic field: The primary known hazard associated with exposure to a static high magnetic field is that the magnet exerts a strong force on ferromagnetic objects. Metallic objects that are entered the magnetic field can accelerate into the magnet potentially causing damage

to the magnet or persons in the magnet room. In addition, implanted metallic objects can be displaced. MRI may not be appropriate in the presence of the following conditions: cardiac pacemaker; metal fragments in eye, skin, body; mechanical heart valve replacement; brain clips; venous umbrella; being a sheet-metal worker or welder; aneurysm surgery; intracranial bypass; renal, aortic clips; middle ear, eye, joint, or penile implants; joint replacements; hearing aid; neurostimulator; insulin pump; intra-uterine device (IUD); shunts/stents anywhere in the body; metal mesh/coil implants; metal plate/pin/screws/wires anywhere in the body, any other metal implants; permanent eyeliner or permanent artificial eyebrows. All participants will be initially screened first via the online screen before enrollment, then using the CMRR Safety Screening Form at the day of MR scanning. The CMRR Safety Screening Form will not be submitted, as further updates are rolled out by the Center for Magnetic Resonance Research. The study will use the published version of the form on the website (https://www.cmrr.umn.edu/policies/). Participants that do not pass screening will not enter the CMRR where magnetic fields exist.

b) Radiofrequency pulses impart small amounts of energy into the participant. No ionizing radiation is used with MRI. Because the pulse sequences to be used fall within FDA guidelines and will not be operated outside of safe limits, we do not expect any hazard associated with power deposition. To prevent inadvertent application of significant energies which may result in heating, the scanning systems include monitoring with both a hardware and a software monitor. Participants will be instructed to report any heating sensation immediately, and that the scan could be stopped at any time if this occurs.

c) Peripheral nerve stimulation from rapidly switching magnetic fields (dB/dt) during the scanning procedure may occur. As a result, the participant may experience muscle twitching or tingling sensations lasting seconds to minutes. This is considered to occur seldomly. Participants are instructed that if twitches do occur, they should immediately inform the operator. This would be a short-lived side effect and reversible.

d) Acoustic noise: MR imaging creates acoustic noise because of pulsing currents through the gradient coils within the magnetic field. A repetitive tapping sound occurs as a result. Ear plugs are provided to the participant to prevent hearing damage and provide comfort

e) Claustrophobia: Some people undergoing this procedure become anxious and afraid when in closed spaces. Participants will be instructed that they can stop procedure at any time. The MRI technologist will be in communication with the participant during the scan and ask them how they are doing. In addition, participants will be given a squeeze ball to communicate an urgent need or concern. Participants are screened for claustrophobia before enrollment.

f) Anatomical abnormalities revealed: There is a possibility that the MRI

scan would reveal unknown and unlooked for abnormalities such as a cyst, vascular abnormality or a tumor. The scan results will be routinely sent to a radiologist for review. If an abnormality is uncovered, the participants are informed of the results of the radiology review and are encouraged to follow up with their physician. We would provide participants physicians with a copy of the imaging data that we collect upon request.

g) Dizziness and nausea: Dizziness and nausea are rare though may occur if the participants head moves around while they are inside the magnet. If this occurs, this would be acute and reversible, and should resolve within a few minutes without intervention.

6. Participants under the age of 18 will provide assent along with parental consent. The potential benefit for those engaging in NSSI, aged 16-24, from the insight gained because of this study outweighs the risks of this study. Risk is minimized by using dosages of NAC determined to be safe from previous research. Study procedures are minimal risk, and staff will be appropriately trained for all study procedures. Checklist HRP-416 is provided.

7. Email Risks: Participants will be able to opt in to communicating with study staff via unencrypted email to arrange their appointments and receive study instructions. There are risks associated with email communication, and these risks increase when the emails are sent without an encryption service. Risks of sending or receiving emails without encryption include, but are not limited to:

   a) Others can intercept messages

   b) If messages are sent or received on an employer-owned device, the employer may have the right to save and read the messages. The internet or cell-phone provider may also have the right to save and read email messages.

   c) A copy of the message may be saved on a device or computer system, even if it is deleted.

   d) If an email address is not typed correctly, it can be sent to the wrong person

   e) Emails can spread computer viruses.

   f) Others may be able to access messages on devices that were lost, stolen, or thrown away. If a user changes emails without notifying study staff, they may miss communications.

8. Text communication Risks: Participants may opt in to communicating with study staff via texting a University-owned cell phone to arrange study appointments and receive other study updates. There are risks associated with text communication, including but not limited to:

   a) Others can intercept messages

   b) If messages are sent or received on an employer-owned device, the employer may have the right to save and read the messages. The internet or

cell-phone provider may also have the right to save and read messages.

c) A copy of the message may be saved on a device, even if it is deleted

d) If the phone number is not typed correctly, it may be sent to the wrong person

e) Others may be able to access messages on devices that were lost, stolen, or thrown away

f) If a user changes phone numbers without notifying study staff, they may miss communications.

Participants will be notified that the phone will only be monitored during business hours (Mon-Fri 9AM-5PM), unless they have an appointment scheduled outside of those hours. They will be informed that they should not use this phone as an emergency contact number as well. Texts that are sent to the study phone will be regularly deleted, unless they are found to be significant to the participant's study record or safety. In this case, screen shots of the texts may be saved, sent to the study email address, and redacted to protect privacy. Please refer to Section 18.0 Confidentiality for redaction procedures. Study staff will not store the participant's name in the phone's contact book. Their number will be saved with their study ID code so study staff may identify them during conversations; once the participant completes participation in the study, their contact will be deleted.

Participants do not have to opt in to text communication in this study. If they change their mind about communicating via text, they can notify staff at any time about their communication preferences. If the participant would like to start using text during the study, they will need to sign a text communication consent form, discussed in Section 11.2 Informed Consent Forms.

7.1 Specification of Safety Parameters

Participants will be asked about their health at the mid-study visit (Day 14) and final study visit (Day 28-32). Participants will be instructed to immediately inform the study team if any serious concerns arise with their health during the study.

The Side Effect Checklist (given at baseline, Week 1, Week 2, Week 3 and Week 4) will be used to assess NAC tolerability. If the side effect is marked as "1" (mild, does not interfere with functioning), nothing will be done. If the side effect is marked as "2" (moderate, some interference with functioning), or "3" (severe, functioning is significantly impaired because of side effects), and this rating denotes an elevation from baseline, and is considered to be at least possibly related to study intervention by the Principal Investigator and/or the Independent Clinical Monitor, we will discuss with participant (and, for adolescents under 18, the parent or guardian) to further assess the participant's impairment from the issue and make a joint decision about the risks versus benefits of staying at the same dose versus decreasing the dose. This determination will consider the frequency and the level of impairment associated with the side effect.

The PHQ-9, the BDI-II and the BSSI (given at baseline, Week 1, Week 2, Week 3 and Week 4) will all be used to assess suicide risk. If the participant indicates elevations in suicidal thinking (e.g. marks a “2” on the question about suicidal thinking on the BSSI) this will trigger a deeper safety assessment as described below.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

The weekly assessment for side effects and suicide risk assessment is based on recommendations from the FDA’s 2004 recommendations that adolescents be seen weekly in the first month of antidepressant treatment to allow for suicide risk assessment.

The study team will review the results of the weekly questionnaires (side effect checklist, ISAS-SLV, PHQ-9) on a weekly basis. In addition, if the participant responded a “2” to any of the following questions on the BSSI: 9, 12, 13, 15, or 16 (these responses correspond to the statements "I cannot keep myself from committing suicide”, "I have a specific plan for killing myself", "I have access or anticipate having access to the method that I would choose for killing myself and also have or shall have the opportunity to use it", "I am sure that I shall make a suicide attempt", and "I have almost finished or completed my preparations for committing suicide,” respectively), this will immediately trigger an automatic email to the study team with the item response and the participant ID, alerting them to contact the participant as soon as possible to assess safety and engage in safety planning. Similarly, if the participant responded a “3” to any question on the side effects checklist, the study team will receive an email prompting them to contact the participant as soon as possible.

The study team will follow up with all participants who rated side effect items as a “moderate” or “severe” to further assess the impact that these issues are having on their functioning. The study team will also follow up with participants or who spontaneously raised concerns about their health. Side effects or other health changes that are confirmed by the research team to be significantly impacting functioning (e.g. interfering with school, work, requiring treatment) will be labeled as adverse events (AEs). All adverse events will be reviewed by the Independent Clinical Monitor who will assist the PI in determining relatedness, whether the AE constitutes an Unexpected Problem (see below) and what corrective measures should be taken (e.g. decrease dose, discontinue treatment, withdraw participant.) The study team will log adverse effects including their timing, relatedness to the study drug, corrective measures taken, and resolution.

7.3 Adverse Events and Serious Adverse Events

An adverse event (AE) is any untoward medical occurrence in a subject during participation in the clinical study or with use of the experimental agent being studied. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.), or any combination of these regardless of relationship to participation in the study.

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect

An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. A suicide attempt will be considered an SAE.

Unanticipated problems are defined as any incidence, experience, or outcome that is:

- Unexpected (in terms of nature, severity, or frequency) given the information provided in research-related documents and the characteristics of the subject population being studied;
- Related or possibly related to participation in the research; and
- Suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

Adverse Events, Unanticipated Problems and Serious Adverse Events will be recorded by study staff throughout the study occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. These will be recorded in an Excel document and kept on file in an electronic secure UMN Box folder for the study. There will be no identifying information recorded in this document and subjects will be identified by subject ID number (GRID number). In addition to whether AE was UP or SAE, details of event, and date of occurrence, we will also label organ system where it occurred (general, psychiatric, nervous system, etc.), severity, expectedness, relationship to study, if it was reported and date of reporting if so, day of trial it occurred (i.e. 1-32), if resolution/stabilization occurred and date of resolution/stabilization. All adverse events will be reviewed with the assigned Independent Clinical Monitor to assist in characterizing the adverse event (e.g. relatedness to study drug, determining if it should be classified as an Unexpected Problem), whether the new information warrants a change to the protocol or consent form (e.g. description of risks) and whether a dose change or participant withdrawal is indicated.

At each study visit (Days 1, 14, and 28 - 32) study staff will inquire about the occurrence of AE/SAEs since the last visit. If AE is ongoing and resolution of AE

has not occurred at time of reporting by subject, study staff will assess resolution/stabilization 1 week later via phone contact. Study staff will cease to assess previous AE of a participant 2 weeks after last day of study participation by that participant.

Safety review by Independent Monitoring Committee will occur if resolution of AE has not occurred 2 weeks after discontinuing study drug (due to study completion or dropout) and AE is considered to be related to study intervention (possibly, probably or definitely) by study PI.

7.4 Reporting Procedures

The Study PI, the Independent Clinical Monitor and the Independent Monitoring Committee will be responsible for determining whether an SAE is expected or unexpected. An adverse event will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the intervention.

Incidents or events that meet the OHRP criteria for unanticipated problems require the creation and completion of an unanticipated problem report form. OHRP recommends that investigators include the following information when reporting an adverse event, or any other incident, experience, or outcome as an unanticipated problem to the IRB:

- Appropriate identifying information for the research protocol, such as the title, investigator's name, and the IRB project number;
- A detailed description of the adverse event, incident, experience, or outcome;
- An explanation of the basis for determining that the adverse event, incident, experience, or outcome represents an unanticipated problem;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the unanticipated problem.

To satisfy the requirement for prompt reporting, unanticipated problems will be reported using the following timeline:

- Unanticipated problems that are serious adverse events will be reported to the Independent Monitoring Committee, and NCCIH within 7 days of the investigator becoming aware of the event.
- Any other unanticipated problem will be reported to the Independent Monitoring Committee, and NCCIH within 14 days of the investigator becoming aware of the problem.

All unanticipated problems should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and OHRP within one month of the IRB's receipt of the report of the problem from the investigator.

The following will be reported to Advarra IRB, the external IRB overseeing this

research study, within 10 days:

- Unanticipated problem that adversely affects the risk/benefit ratio of the study, or rights, safety, or welfare of the participants or others, or integrity of the study. (Advarra IRB form: Safety Information and Unanticipated Problem Report)
- An adverse event that is serious, unanticipated, and [study] related. (Advarra IRB form: Safety Information and Unanticipated Problem Report)
- Any change from Board-approved protocol that adversely affects the risk/benefit ratio of the study, or rights, safety, or welfare of the participants or others, or integrity of the study. (Advarra IRB form: Safety Information and Unanticipated Problem Report)
- Any prospective request for an intentional deviation from the IRB approved protocol except when necessary to eliminate an apparent immediate hazard to a participant. (Advarra IRB forms: Copy of Sponsor approval and Prospective Waiver/ Exception Request Form)
- A complaint associated with the study regarding an alleged breach of the rights, safety, or welfare of the participants or others, or integrity of the study. (Advarra IRB form: Safety Information and Unanticipated Problem Report)
- Any adverse finding issued to, or enforcement action taken against, the PI. (Advarra IRB forms: Copy of the adverse audit results, enforcement action, etc. and Safety Information and Unanticipated Problem Report
- Any significant problems, violations, research-related accidents and illnesses must be reported. Spills or accidents in BL2 laboratories resulting in an overt exposure must be immediately reported. (Advarra IRB form: Safety Information and Unanticipated Problem Report)

Investigators in the Department of Psychiatry conducting clinical drug trials are required to notify The Office of Ombudsman for Mental Health and Developmental Disabilities (OMHDD) within 24 hours of a U of M research participant's death or serious injury. This includes suicide attempt. The Ombudsman Reporting Transmittal Form is used by HRPP and the University of Minnesota Principal Investigator to report to OMHDD pursuant to Minnesota Statute 245.92 and 245.94. Reports of death or serious injury of locally enrolled study participants in psychiatry clinical drug trials must use the Transmittal Form as the cover page to the applicable report form available on the OMHDD website.

Reporting to Advarra IRB, the University of Minnesota IRB, the OMHDD, the NCCIH and the Independent Monitoring Committee will be performed by the Research Coordinator (Siddhee Sahasrabudhe), Regulatory Specialist (Kathleen Thaemlitz) or the Principal Investigator (Kathryn Cullen).

SAEs that are unanticipated, serious, and possibly related to the study intervention will be reported to the Independent Monitoring Committee, IRB, FDA, and NCCIH in accordance with requirements. For the IND:

- 7-day IND Safety Report (unexpected fatal or life-threatening AEs related to the intervention); a copy of the report sent to the FDA will be submitted to the NCCIH Program Officer and Independent Monitoring Committee within 24 hours of FDA notification.
- 15-day IND Safety Report (any other serious and unexpected AE related to the intervention); a copy of the report submitted to the FDA will be submitted to the NCCIH Program Officer and Independent Monitoring Committee within 24 hours of FDA notification.
- All other AEs documented during the course of the trial will be reported to NCCIH on an annual basis by way of inclusion in the annual report and in the annual AE summary which will be provided to NCCIH and to the Independent Monitoring Committee. The Independent Monitoring Committee Report will state that all AEs have been reviewed.

Pregnancy is an MRI contraindication and is a part of exclusion criteria. If a potential subject is pregnant, they will not pass screening as a result. If a subject becomes pregnant while enrolled in the study, they will then no longer meet criteria, be ineligible and be removed from the study. Since we conduct a pregnancy test prior to MRI scanning, this will be picked up before MRI scanning. This will be recorded in our enrollment log if occurs. Since there are no known risks associated with NAC and pregnancy, study staff will not report this immediately, but will be reported as part of the enrollment log when reporting to Advarra IRB continuing review, as well as any other reporting that includes the enrollment log (Independent Monitoring Committee, FDA, NCCIH).

7.5 Follow-up for Adverse Events

If AE is still present at the time it is reported, it will be re-assessed one week later either in person, via online survey or via phone. If the AE is still present 2 weeks after last day of study participation, it will be considered stable, and we will not continue assessments. Safety review by Independent Monitoring Committee will occur if resolution of AE has not occurred 2 weeks after discontinuing study drug (due to study completion or dropout) and AE is considered to be related to study intervention (possibly, probably or definitely) by study PI and the Independent Clinical Monitor.

7.6 Safety Monitoring

The Principal Investigator has appointed an Independent Monitoring Committee for this research study. The members include the following: Dr. David Brent, M.D., a child psychiatrist with expertise in adolescents at risk for self-harm from University of Pittsburgh; Dr. Manpreet Singh, a child psychiatrist from Stanford University with expertise in adolescent depression and clinical trials; Dr. Bin Cheng, an Associate Professor of biostatistics from Columbia University Medical Center. The Committee will meet for the first time 6 months after the start of study enrollment, and then again one year later. The PI has also assigned Dr. Gamze Balci Camsari to act as the Independent Clinical Monitor to assist the PI in (1) assessing and characterize adverse events (2) determining whether study drug should be reduced or discontinued, and (3)

determining whether the participant should be withdrawn from study procedures.

8. INTERVENTION DISCONTINUATION

The intervention will be discontinued in the following circumstances:

- If the participant is unable to tolerate the study medication due to side effects or allergic reaction, even after decreasing the dose, as determined by a conversation between the PI (or delegate) and the participant.
- If the participant elects to no longer take the study medication.
- If the participant becomes actively suicidal with intent and plan, and cannot agree to a safety plan engaged by study team (see Supplement IV).
- If the participant or parent of minor participant withdraws consent.
- If the participant becomes pregnant.
- If the IMC or the Medical Monitor recommend participant withdrawal due to safety concerns.

Discontinuing the intervention will also be considered in the following circumstances:

- If a participant engages in NSSI with significant tissue damage that requires urgent/emergency medical care, study staff will assess safety of participant and engage participant (and parents of minors) in a safety conversation to discuss how to increase safety in the home, whether hospitalization is needed, and whether continuing in the study is appropriate (See Supplement IV). If it is deemed safe by study staff with respective input from NCCIH, FDA, Advarra IRB and/or IMC when necessary and participant (and parents of minors) desire to continue, remaining study visits will be completed.
- If a participant fails to adhere to protocol requirements, study staff will assess safety of participant with respect to protocol deviation(s) and engage participants (and parents of minors) in conversation to discuss their safety and if continuing in the study is appropriate. If it is deemed safe by study staff with respective input from NCCIH, FDA, Advarra IRB and/or IMC when necessary and participant (and parents of minors) desire to continue, remaining study visits will be completed.
- Changes to psychiatric medications during the study are strongly discouraged, and we will ask participants to promptly notify us of any changes. If such changes occur, the Principal Investigator and co-investigators Coles, Kartha or Cloyd will review the relevant information and potential interactions. If it is determined that the new medication(s) raise potential safety concerns while being taken with NAC, the participant will be advised to stop taking study drug and still complete study visits as planned. Otherwise, the participant will continue. These changes will be documented and incorporated into sensitivity analyses as described in section 9.6.

For all above withdrawal circumstances, participant contribution to subsequent data

collection will depend on safety considerations, participant interest and the adherence of each participant (Section 5.3).

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

The goals of this study are to test whether significant change can be found in candidate biological signatures after 4 weeks of oral NAC. Three groups are used to compare pre-post treatment changes in those who received NAC 5400mg/day, 3600mg/day, or PBO.

Primary Hypothesis:

1) NAC high dose and/or NAC low dose (but not PBO) will increase cortical GSH by at least 5%

Secondary Hypotheses:

1) NAC high dose and/or NAC low dose (but not PBO) will decrease cortical GLU by at least 5%
2) NAC high dose and/or NAC low dose (but not PBO) will increase blood GSH/GSSG by at least 50%
3) NAC concentrations (using data from both dose groups) will be correlated with clinical and neuroimaging outcomes.
4) NAC high dose and/or NAC low dose (but not PBO) will increase resting-state functional connectivity between amygdala and insula
5) NAC high dose and/or NAC low dose (but not PBO) will increase ACC GABA.
6) NAC high dose and/or NAC low dose (but not PBO) will increase antioxidant protein levels (catalase and heme oxygenase-1 [HO-1]).
7) Higher-dose NAC will show larger changes in all of the biological signatures listed above compared to lower-dose NAC
8) While both NAC doses will be acceptable to most patients, lower-dose NAC will be better tolerated than higher-dose NAC.

Primary outcome measures:

1) GSH concentration in ACC

Secondary outcome measures:

1) Blood GSH/GSSG (the reduced-to-oxidized GSH ratio in blood)
2) Glu concentrations in ACC
3) Tolerability of NAC as measured using a side effects checklist
4) Pharmacokinetic analysis of NAC and its metabolites, CYS and GSH
5) Antioxidant protein levels (catalase and heme oxygenase-1 [HO-1]) for which

previous studies have shown increases following NAC treatment.

6) GABA concentrations in the ACC. Our pilot study found a significant increase in GABA levels after 28 days of oral NAC (6000 mg/day) (Coles et al. 2018).
7) Functional connectivity between amygdala and insula. Our pilot study showed that after treatment with oral NAC (3600 mg/day), participants with NSSI showed an increase in amygdala functional connectivity in a cluster that included the bilateral insula.

9.2 Sample Size and Randomization

The sample size (n=12 per group) was selected based on calculations on biosignature data from the oral NAC pilot study in adults with either Parkinson's disease (PD; n=5) or healthy controls (HC; n=3). Using variability estimates from this study, we found that with groups of 11 (assuming one drop-out per group), we would have 80% power to show a significant (alpha=0.0167, Bonferroni corrected for 3 pairwise comparisons) effect of NAC high vs. NAC low vs. PBO on our primary outcome of brain GSH if the variability is similar to healthy controls seen in our pilot work (detectable mean group difference in pre-dose to post-dose percent change of 2.95%, expected mean group difference ~4.83%). The data collected in the R61 will serve to provide the variability information needed to design a future, confirmatory clinical trial.

Treatment Assignment Procedures

Randomization and Double-Blind: Participants who meet criteria for entry into the study (based on the online screen and the clinical interview) and who provide consent will be randomized to active NAC low dose versus NAC high dose versus PBO (1:1:1). We will implement an adaptive randomization procedure called minimization. The purpose of the procedure is to ensure that the 3 groups are similar with respect to key demographic and clinical variables such as age, NSSI severity and medication use. See Section 6.2.2 for a complete description of the randomization procedure.

All participants, family members, and study staff (including the study statistician) will be blinded to treatment assignment. The research pharmacy, IDS, will be the only unblinded entity. See Section 6.2.2 for a complete description of the blinding (and unblinding) procedures.

9.3 Definition of Populations

The Intent to Treat population will include all those who completed both baseline and 4-week MRI scans and blood draws, even those who discontinued the study medication due to intolerability or other reasons. The Per-Protocol analysis population will consist of only those subjects who completed all procedures.

9.4 Interim Analyses and Stopping Rules

The following safety findings would prompt temporary suspension of enrollment and

study intervention until a complete safety review is convened:

- Suicide attempt in greater than 20% of participants that have received study intervention beginning when at least 10 participants have received study intervention.
- Suicide attempt in greater than 30% of participants that have received study intervention when between 1 and 9 participants have received study intervention.
- SAE in greater than 30% of participants to receive study intervention
- SAE occurrence in first 3 participants to receive study intervention
- AE indicating anaphylaxis to study intervention in at least 30% of participants to receive study intervention beginning when 10 participants have received intervention
- Suicide completion in at least 2 participants to receive study intervention
- Anything else deemed a safety risk to participants by the investigator or the IMC

"Study intervention" refers to any or all 3 of the treatment groups. The following safety findings would prompt an ad hoc safety review without suspension of enrollment and study intervention:

- NSSI frequency in past month increase of 100% in any single participant as determined by SITBI (question 148), comparing Day 1 and Day 28 – 32 assessments.
- Average NSSI frequency in past month increase of 50% during study enrollment as determined by SITBI (question 148, NSSI section, "How many times in the past month?") comparing Day 1 to Day 28 - 32 measurement beginning when 5 participants have received study intervention.
- Suicide completion in 1 participant to receive study intervention

A safety review will be carried about by the Independent Monitoring Committee and its occurrence would be reported to Advarra IRB and NCCIH. Safety findings such as those listed above will be presented to Independent Monitoring Committee to review the events by blinded group to determine whether there are statistical as well as clinical concerns. The objective of the safety review is to determine whether the study or intervention (NAC) for an individual or for the study cohort should continue per protocol, proceed with enhanced monitoring, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrollment is a potential outcome of a safety review.

Outside of safety reviews, no interim analyses are planned for this study.

9.5 Outcomes

9.5.1 Primary Outcome

1) GSH concentration in ACC voxel. Resolution of reduced versus oxidized GSH using spectroscopy is not possible; GSH concentrations as measured by MRS represent primarily reduced, intracellular GSH. Collected during neuroimaging procedures at Visit 2 and Visit 4.

9.5.2 Secondary Outcomes

1) Blood GSH/GSSG, the reduced-to-oxidized GSH ratio in blood. Performed on blood collected at Visit 2 and Visit 4.
2) Glu concentrations in ACC voxel. Glu can be reliably separated from Gln at 7T. Glu concentrations as measured using a side effects checklist. Collected during neuroimaging procedures at Visit 2 and Visit 4.
3) Tolerability of NAC as measured using a side effects checklist. Collected at Visits 2, 3, 4 and through on-line measures between visits.
4) Pharmacokinetic analysis of NAC and its metabolites, CYS and GSH. Performed on blood collected at Visit 4.
5) Antioxidant protein levels (catalase and heme oxygenase-1 [HO-1]) collected at visits 2 and 4
6) GABA concentrations in the ACC collected at visits 2 and 4
7) Functional connectivity between amygdala and insula, data collected at Visit 2 and 4

9.6 Data Analyses

To address the study objectives, we will quantify the within-person percent change in proposed primary biological signature: brain GSH. We will fit a general linear model with brain GSH as dependent variable with group (NAC high vs NAC low vs PBO) as the predictor variable of interest. Since we apply a minimization procedure to ensure that groups are similar on key demographic and baseline clinical variables (age, baseline NSSI severity, presence of concurrent psychotropic medication), as is typically done in a large clinical trial we will not include covariates. Should the primary outcome values be highly skewed, we will use non-parametric tests instead to compare groups. We will use the standardized Wilcoxon test statistic with the Dwass, Steel, Critchlow-Fligner (DSCF) multiple comparison analysis. This analysis takes each pair of groups, one pair at a time (high NAC vs. low NAC; high NAC vs. placebo; low NAC vs. placebo), computes a Wilcoxon test statistic to compare the two groups in that pair, and obtains a pvalue that is analogous to Tukey's control of family-wise Type I error (the test statistic is scaled to Tukey's studentized range distribution) (Hollander and Wolfe, 1999). This analysis to compare the 3 treatment groups on the primary outcome will adjust type I error using Tukey’s procedure for the 3 pairwise comparisons among the 3 groups. Tukey’s procedure is less conservative than the Bonferonni adjustment used in the sample size/power

calculation, hence we expect slightly higher power than planned. We will quantify the effect size of each group's change to determine next steps with the research.

To assess the robustness of our conclusions about the primary outcome to participants whose medical profile changes during the course of active treatment in our study, we will conduct sensitivity analyses. For example, if participant(s) need to have a change in their non-study medications during our study, they will remain in the study but the changes in medications will be noted in the study database. We will repeat our primary analysis excluding such people to see if our conclusions remain the same. We note, however, that this analysis is not protected from bias by the randomization.

To address the secondary study objectives of exploring additional potential biosignatures, we will fit similar general linear models with each of the additional outcomes (brain Glu, blood GSH/GSSG, antioxidant protein levels, GABA concentrations, amygdala-insula functional connectivity) as dependent variables, with group (NAC high vs NAC low vs PBO) as the predictor variable of interest, again without including covariates to preserve power. These secondary analyses will use Holm's step-down Bonferroni type I error correction to adjust for both multiple comparisons among groups and multiple testing across the several secondary outcomes.

Data analysis: To address the secondary outcome of NAC PK, NAC and GSH concentration-time data will initially be analyzed by non-compartmental methods. WinNonLin Phoenix (Pharsight®) will be used to calculate the partial NAC area under the curve (AUC_0-2h) using the linear trapezoidal rule and maximum concentration (Cmax) . The AUC_0-2h and Cmax for GSH will also be calculated. Descriptive statistics of the PK parameters will be determined. Drug concentrations and other PK variables will be correlated with degree of change in biological signatures.

To address the secondary objective of describing the tolerability of oral NAC at the proposed study doses, we will quantify rates of side effects and other adverse events and compare rates between groups using Poisson, survival, or other rate-based models as appropriate for each event type.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Information will be collected from participants by Principal Investigator, Kathryn Cullen, MD, Co-Investigator Bonnie Klimes-Dougan, PhD, Research Coordinator, Siddhee Sahasrabudhe, and University of Minnesota Psychology graduate students under the supervision of Drs. Cullen and Klimes-Dougan. All study staff collecting information will be blinded. Information will be recorded directly onto paper copies of measures or directly into REDCap as source data. Measures recorded on paper copies will be transferred to REDCap or secure Box, though source data and

confidential information will be kept in a locked cabinet in a locked room.

10.2 Data Management

Storage and Access: Data will be collected using a combination of paper and electronic measures. Data will be stored and administered electronically in a REDCap database managed by the University of Minnesota Academic Health Centers Information Systems (AHC-IS) (https://redcap.ahc.umn.edu) and a HIPAA compliant secure UMN Box folder identifiable by subject ID. Demographic information containing PHI, such as age, date of appointments, race, age, etc., will be entered directly, and stored, via an electronic measure, Demographics Form, into REDCap. Neuroimaging data will be stored on secure servers of the Center for Magnetic Resonance Research (CMRR) and will not contain any PHI, identifiable only by subject ID (participant specific) and procedure number (scan specific).

Access to the REDCap project and the UMN Box folder will be granted by Research Coordinator (Siddhee Sahasrabudhe) and PI (Kathryn Cullen) as "owners" to study staff access as "editors". REDCap and secure Box are accessible by UMN staff, faculty and students via UMN username and password managed by AHC-IS. The built-in audit trail in REDCap allows administrators to be able to determine all the activity and all the data viewed or modified by any given user in REDCap.

The following measures will be collected and stored for future analysis: Demographic information including sex, age, race, ethnicity, diagnosis and four factor social status via the Demographic Form on REDCap, WASI-II, Edinburgh, PHQ-9, DTS, CTQ, BSS, side effects checklist, SITBI, ABUSI, BDI-II, DSHQ-M, ISAS-Lifetime, Anti-Depressant Medication form, Ongoing Medication Use and Changes: Initial Visit, Ongoing Medication Use and Changes: Subsequent Visits, and ISAS-SLV.

Release/Sharing: For data releases, request for sharing will be made to the PI, Kathryn Cullen and/or co-investigator, Bonnie Klimes-Dougan and granted on an individual basis. Participants that complete MRI scans will be offered a copy of a portion of their scan on disc. A written report detailing information gathered at the clinical assessment (MINI-KID or MINI), including psychiatric diagnosis, will be shared with participants provider(s) of choice upon receiving signed release of records from the participant. Additional specific measures gathered will be shared with participants providers upon signed request. General findings after data analysis will be shared with all participants in the form of a mailed letter.

Specimen blood samples will be processed for analysis and then stored at the Center for Orphan Drug Research (CODR). These samples will be stored for at least 5 years to allow for follow-up tests that may be required, such as if new tests become available, or if manuscript reviewers request additional analyses.

10.3 Quality Assurance

10.3.1 Training

Investigator and study staff will operate under the standard operating procedures (SOPs) of the University of Minnesota human research protection program found in

the toolkit library at https://research.umn.edu/units/irb/toolkit-library/standard-operating-procedures. All study staff on this study will be delegated particular tasks as indicated by the Delegation of Authority Log kept on site. Study staff will be trained on each updated protocol and recorded on a protocol training log.

All study staff will receive HIPAA training via University of Minnesota and Good Clinical Practice training via the Collaborative Institutional Training Initiative (CITI), as required by the UMN Department of Psychiatry. Study staff consenting candidates will each perform a training program documented and signed-off on-site before they perform said activities.

Training oversight

1) Delegation of Authority log will show which personnel are delegated to which study procedures.
2) Protocol training log will show all personnel up-to-date on current protocol versions.
3) Psychological assessment training will be conducted by Bonnie Klimes-Dougan and reflected in the DoA and Psychological Assessment Training log.
4) IV catheter placement and phlebotomy will be conducted by trained Fairview/M Health staff

10.3.2 Quality Control Committee

Quality Assurance (QA) Reviewer will review monthly report of adherence to the treatment protocol and adherence of participants. QA Reviewer will be lab associate not working directly on this project.

10.3.3 Metrics

Quality assurance of metrics will be as follows:

Spectroscopy measures: To ensure identical voxel positioning in the pre- and post-NAC scans, we will utilize the AutoAlign feature on Siemens by saving subject specific protocols at the baseline. Acquisition methods and evaluation of the CSF contribution to the ACC voxel will be as described in our previous work (Terpstra et al 2016, van de Bank 2015). Methods to assess the reliability of metabolite concentrations based on Cramér-Rao lower bounds will be identical to those described in our previous work (Terpstra et al, 2016).

10.3.4 Protocol Deviations

Protocol deviations will be recorded by study staff in a protocol deviation log, with attached memo if further explanation is needed. Protocol deviation logs will be reviewed every 6 months by a study monitor from the Independent Monitoring Committee. This monitor will also review study documentation to assure the protocol deviation log is up-to-date upon review.

Any unapproved protocol deviations (an accidental or unintentional change to the IRB-approved protocol) that, in the investigator's judgment, potentially caused harm

to subjects or others, indicates that the subjects or others are at an increased risk of harm, or has adversely impacted data integrity, will be reported to the IRB promptly and no later than 2 weeks (10 business days) from the time of the identification of the unplanned or unintentional protocol deviation/violation.

There are many unplanned or unintentional violations/deviations or changes in study status that do not cause harm, place subjects at increased risk of harm, or adversely affect data integrity. The IRB does not require that these minor violations/deviations be reported. Examples of minor violations/deviations that do not need to be reported may include the following:

- Out of window visits
- Study procedures conducted out of timeframe
- Subject failure to initial each page of the ICF (as applicable)
- Subject failure to return subject materials (e.g., diaries, journals, etc.).
- Administrative hold on a study not related to safety issues

Examples of accidental or unintentional protocol violations/deviations that must be submitted to the IRB include:

- Changes necessary to eliminate apparent immediate hazards to the subject
- Failure to document informed consent
- Informed consent obtained after initiation of study procedures
- Enrollment of a subject who did not meet all inclusion/exclusion criteria
- Performing study procedure not approved by the IRB
- Failure to report serious adverse event to the IRB and/or sponsor
- Failure to perform a required lab test that, in the opinion of the investigator, may affect subject safety or data integrity
- Drug/study medication dispensing or dosing error
- Study visit conducted outside of required timeframe that, in the opinion of the investigator, may affect subject safety
- Failure to follow safety monitoring plan
- Missing or unreturned investigational product

10.3.5 Monitoring

Data integrity monitoring will be conducted at outset, every 6 months, and upon study closing by the NCCIH. The monitor will review an on-site regulatory binder as well as participant folders to ensure compliance to the protocol, SOP and other regulatory requirements. The monitor will review consent forms, completion of source documents and case report forms, and appropriate documentation for protocol deviations, personnel changes, staff training, and adverse events, etc. The monitor

will have access to and review all necessary documents at a set appointment time. Monitoring reports will indicate enrollment numbers, select changes since the last monitoring visit and any unresolved queries. The research staff will be expected to resolve all queries, and notify monitor of these resolved queries, within 10 days of receiving the monitoring report.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and all consent documents associated with this study protocol (parent consent, adult consent, assent form) and any subsequent modifications will be reviewed and approved by NCCIH, Advarra IRB and the FDA for oversight of the study. The consent forms will be reviewed and approved separate from this protocol document.

11.2 Informed Consent Forms

The consent process will take place at Visit 1, before the clinical assessment. Participants will receive copies of the consent / assent (if applicable), email authorization and HIPAA forms via email or USPS mail at least 24 hours before this appointment. The initial consent process will take place with research staff in a previously reserved room within the Ambulatory Research Center (ARC) at the University of Minnesota Department of Psychiatry (F212) Fairview Riverside West Building or at the Research in Adolescent Depression Lab at the University of Minnesota Medical School in-person or through video conferencing. During the consent process study staff will review the consent forms highlighting the most salient points such as study title, purpose, procedures, risks, benefits, alternatives, confidentiality, research related injury, voluntariness, and any additional signing/initial blocks such as permission to re-contact in the future and share data outside of this research study. Talk-back method will be used during the consent visit to ensure potential participant and/or their parent/guardian (if applicable) are understanding these points. In addition, an approved version of the UCSD Brief Assessment of Capacity to Consent specific to this study will be used with all participants during the consent process. Potential participants that are not able to complete the UBACC or score less than 2 points on each question will not be consented and enrolled into the study. If a potential participant scores 0 or 1 on any question the information pertinent to that question will be reviewed and discussed with the participant. If the potential participant is then able to answer the question in full and research staff determines the potential participant fully understands the material, they will be scored the 2 points and then consented and enrolled (assuring all answers are scored 2 points). In addition to University and department mandatory consent training for appropriate research staff, all new staff will be trained in consent for this research study including observing the consent process, mock consent and observed for consent process. Throughout the course of the research study, participants will be gauged of their comprehension of scheduled procedures as well as comfort and willingness to complete these procedures by staff at each visit/procedure. Consent will be documented in ink or through electronic signatures by signature of

parent/guardian on Parental Consent Form (if applicable), under 18 participants on Assent Form (if applicable), 18 or over participants on Adult Consent Form (if applicable) and completed UBACC form (all participants). Research staff obtaining consent will sign (ink or through electronic signatures) each of these applicable forms and document the consent process using the ICF Documentation Form.

This study will not enroll non-English speaking participants.

Potential participants will be screened to determine if they meet age requirements (16-24). They will be asked to bring or produce identification in case of video conferencing and checked of their age at the consent process. Potential participants that fail to bring or produce identification with DOB will not be consented and enrolled. Potential participants age 16 or 17 will be consented as children and those age 18-21 will be consented as adults.

For those age 16 or 17, parental permission will be obtained from at least one parent to consent children eligible for the study. Parental permission will be attempted to be obtained from both parents unless one parent is deceased, unknown, incompetent or not reasonably available due to travel or work. Permission will be obtained from someone other than a parent only when that person is a legal guardian of the potential participant. Assent will be obtained and documented from all participants that meet criteria and are willing to consent that are age 16 or 17. Documentation will be obtained by child signing Assent Form, along with research staff personnel obtaining consent.

Research staff will use a study specific version of the UCSD Brief Assessment of Capacity to Consent (UBACC) for all potential participants (16-24) at the consent visit.

Participants will be able to opt into receiving unencrypted email communication and from study staff for reminders about appointments or instructions. The participant will not be required to communicate via email, and they may change their mind about email communication at any point during the study by informing the study team of their decision. Participants will also have the choice to opt into using texting communication in this study. Texting would allow study staff to remind participants about their appointments, completing remote assessments, or to provide other study updates. To consent into using texting communications, participants must sign the Text Authorization Form. The paper copy will be scanned onto Box or an electronic signature will be recorded in case the visit is completed through video conferencing.

11.3 Participant Confidentiality

All information and data collected from and about participants during the research study will be de-identified and kept in the participant's folder in a locked cabinet in a locked room accessible by only study staff. Identifiable forms such as screening forms containing DOB and consent forms will be kept in a confidential section of the participant's folder. All research staff and volunteers are required to complete data safety and security and HIPAA training according to University of Minnesota policy. All data electronically stored and transferred will be a limited data set and any

participant PHI stored electronically will be kept secure in Box, accessible by only study staff, and, if transferred, will be encrypted using secure portal such as Box. Confidentiality will be broken in the event of harm or threat to safety of subject is discovered during the research study. Examples of this occurrence may include the following: informing the parent of minor of suicide attempt, suicidal ideation or incidence of self-injury believed by study staff to be safety concern; contacting Child Protective Services in incidents of abuse, neglect or threat; reporting occurrence of abuse or harm, including sexual assault, from peer, adult family member or adult non-family member; walking subject to Emergency Department in event of suicidal intent and plan. See Supplement IV: Procedure for suicide and self-harm risk assessment in research participants.

Participants may opt into sending study staff emails or texts by signing additional authorization forms. In some cases, clinically significant information may be shared with the study team through these messages. In these cases, a member of the study team may save the communication to provide important context for the individual's study case. For emails, the messages will be saved as a PDF on an AHC-IS protected computer. For texts, screenshots of the conversations will be saved and sent to the study-specific email address, and then the images will be saved as PDFs on an AHC-IS supported computer. If these communications need to be shared outside of the study team for any reason, staff will redact any identifying information. Study staff will then redact the PDFs by putting black bars over any identifying information (name, email address, phone number, etc.). Dates and times of messages will be maintained to provide context for the conversation. A secondary staff member will review the PDFs to ensure that all identifying information has been removed prior to sending the messages to anyone outside of the study team.

11.4 Study Discontinuation

This study may be discontinued at any time by Advarra IRB, University of Minnesota IRB/HRPP, NCCIH or FDA as part of their oversight responsibilities, to ensure that research participants are protected and safe from harm.

12. COMMITTEES

The Principal Investigator has appointed an Independent Monitoring Committee for this research study for safety monitoring. There are no other committees affiliated with this research study.

13. PUBLICATION OF RESEARCH FINDINGS

Publications and presentations of the findings that result from this study will acknowledge the funding support of the NCCIH

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15. SUPPLEMENTS/APPENDICES

Supplement I:
A) Original PK sample collection scheme: Blood draw timing and blood processing

To fully characterize the PK profile, we will adopt sparse sampling. At the final visit (Day 28 - 32) we will collect samples before and after their final dose of the study drug, pre-dose and post-dose respectively. In addition, we will collect samples prior to and after their scanning session (i.e. ~60 minutes and ~2-3 hours after dosing, respectively). Additional samples will be collected up to ten (10) times bracketing 15 minutes and 6 hours post-dose. Participants will be separated into two groups and collection times will be staggered between the two groups. The times will be chosen based on prior NAC pharmacokinetics research showing that after oral dosing, NAC plasma concentrations peak levels at about 90 minutes, with a half-life of ~6 hours (Holdiness 1991). Following our standard lab procedure, we will collect from ~1 teaspoon to 1 tablespoon of blood in tubes containing K3EDTA, avoiding hemolysis during collection. The Day 1 collection will be ~1 tablespoon. The first sample on Day 28 - 32 will be ~1 tablespoon and each of the post-dose samples will be ~1 teaspoon spanning ~4-5 hours. Tubes will be placed on ice until processing. Samples will be centrifuged at 4°C, to separate plasma and red blood cells placed on dry ice, and stored in a -86°C freezer. GSH/GSSG and total GSH will be measured in blood using high performance liquid chromatography (HPLC) coupled to a mass spectrometer (MS) as previously reported (Kartha et al. 2015; Holmay et al. 2013a). Total (reduced + oxidized) concentrations of NAC, and CYS will be measured in plasma using a validated HPLC-MS assay (Radtke et al. 2012). To save on cost, we will not measure NAC and CYS levels in the PBO groups. Plasma HO-1 levels will be determined using HO-1 human ELISA kit (Enzo Life Sciences, Farmingdale, NY, USA) as we have done previously (Kartha et al. 2015). Catalase enzyme activity will be measured in red blood cell lysate using Catalase Assay kit (Cayman Chemical, Ann Arbor, MI) as per manufacturer's instructions.

B) Abbreviated PK sample collection scheme: Blood draw timing and blood processing

The ~8h duration of visit 4 may introduce risk to participants due to extended time indoors win a public building during the COVID-19 pandemic. Therefore we will use an abbreviated protocol during the pandemic; the study PI will make the determination of practicing original PK sample collection scheme for visit 4 once it is safe to do so based on the guidance from the University.
The abbreviated PK sample collection scheme is as follows:
At the final visit (Day 28 - 32) we will collect samples before their final dose of the study drug which will be taken at the CMRR. In addition, we will collect samples prior to and after

their scanning session (i.e. ~15 minutes after the first blood draw and ~1.5 hours after dosing, respectively). Following our standard lab procedure, we will collect from ~1 teaspoon to 1 tablespoon of blood in tubes containing K3EDTA, avoiding hemolysis during collection. The Day 1 collection will be ~1 tablespoon. The first sample on Day 28 – 32 will be ~1 tablespoon and each of the post-dose samples will be ~1 teaspoon. Tubes will be placed on ice until processing. Samples will be centrifuged at 4°C, to separate plasma and red blood cells placed on dry ice and stored in a -86°C freezer. GSH/GSSG and total GSH will be measured in blood using high performance liquid chromatography (HPLC) coupled to a mass spectrometer (MS) as previously reported (Kartha et al. 2015; Holmay et al. 2013a). Total (reduced + oxidized) concentrations of NAC, and CYS will be measured in plasma using a validated HPLC-MS assay (Radtke et al. 2012). To save on cost, we will not measure NAC and CYS levels in the PBO groups. Plasma HO-1 levels will be determined using HO-1 human ELISA kit (Enzo Life Sciences, Farmingdale, NY, USA) as we have done previously (Kartha et al. 2015). Catalase enzyme activity will be measured in red blood cell lysate using Catalase Assay kit (Cayman Chemical, Ann Arbor, MI) as per manufacturer's instructions.

Supplement II: Neuroimaging Procedures

All brain imaging will be completed at the CMRR on a 7T whole body Siemens MAGNETOM scanner (Siemens Medical Solutions, Erlangen, Germany) using a standard Nova coil. We will use dielectric padding during the MRS acquisition but remove it for the fMRI acquisition. High-resolution T1-weighted structural data acquisition: A T1-weighted MPRAGE image will be acquired to position the MRS voxel and to facilitate registration of the fMRI data for group analyses. T1 parameters will include: TR = 3000 ms, TE = 3.27 ms, TI = 1500 ms, flip angle 5°, slice thickness=1 mm, number of slices= 176, field of view = 256 x 256 mm2, and matrix size =256 x 256, 4 minutes. A proton density image will be acquired and used to reduce the image inhomogeneity of the T1 weighted image to improve image processing. PD parameters will include: TR = 1410 ms, TE = 3.27 ms, flip angle 5°, slice thickness=1 mm, number of slices= 176, field of view = 256 x 256 mm2, and matrix size =256 x 256, 2 minutes. FreeSurfer (Fischl 2012) will be used to conduct initial processing of the ratio image from the T1 and PD weighted scans and to parcellate the brain into standard regions of interest which will be used for the fMRI analyses. Spectroscopy acquisition and analysis (to obtain primary outcomes GSH and Glu concentrations): Proton spectra will be acquired from the ACC (2.0 x 2.0 x 2.0 cm3). Reproducible voxel placement may be accomplished with anautomated VOI placement tool, when the tool is developed (Park et al 2018 MRM). Acquisition methods and evaluation of the CSF contribution to the ACC voxel will be as described before (Terpstra et al. 2016; van de Bank et al. 2015). Specifically, we will utilize an optimized semi-LASER sequence (Öz and Tkáč 2011) with TE=26ms, TR=5s, 128 transients per spectrum. First- and second-order B0 shims will be adjusted in the ACC voxel using FASTMAP (a fast, automatic shimming technique by mapping along projections) with an echo planar imaging readout (Rolf Gruetter and Tkáč 2000). Next, B1 levels for localization pulses and water suppression in semi-LASER will be adjusted. In addition, we will utilize real time voxel tracking, shim and frequency updates (see section C.1.c). Methods to quantify the neurochemical profiles using LCModel (Provencher 2001), and to assess the reliability of metabolite concentrations based on

Cramér-Rao lower bounds, will be identical to those described before (Terpstra et al. 2016). Functional imaging acquisition and analysis (to obtain secondary outcome amygdala-insula RSFC): Functional data will be acquired using the Human Connectome Project multiband echo planar imaging sequence for 7T (Van Essen et al. 2012; T Vu et al. 2017). Whole brain T2*-weighted functional volumes (85 contiguous slices; TR=1000 ms; TE=22.2ms; flip angle=45o, matrix=208x208mm; voxel size=1.6mm isotropic; multiband factor=5, GRAPPA=2, echo spacing = 0.64ms) will be obtained during rest. Patient will be instructed to keep eyes open while viewing a fixation cross (2 runs at 6 min each). The duration and the choice of fixation cross as the resting condition are selected to optimize reliability (Birn et al. 2013; Patriat et al. 2013). Whole-brain functional connectivity maps of the amygdala will be obtained for each person at each time point using methods described previously (Cullen et al. 2014; Westlund Schreiner et al. 2017). We will extract the mean z-score from anatomically-defined insula regions of interest, to represent amygdala-insula connectivity at each time point. These values will be used in repeated-measures ANOVAs to evaluate change in amygdala-insula connectivity over time.

Supplement III: Identification of Potential Participants

1) For those screened out or enrolled in previous research study, research staff will identify those that may be eligible for this study and contact them. For those enrolled in previous study, we will only contact those that have indicated in previous consent forms an interest to be contacted about future studies.

2) For the University of Minnesota Psychiatry outpatient clinic research participant registry, the outpatient Psychiatry Clinic in the Department of Psychiatry at the University of Minnesota provides new patients the opportunity to indicate whether they are interested in being contacted to hear about research studies or whether they prefer to not be contacted. Access to this contact information is managed by the Department of Psychiatry Clinical Research Recruitment Specialist. The Clinical Research Recruitment Specialist describes research opportunities to patients and provides the contact information of interested patients to departmental researchers conducting studies for which they might be eligible. We are requesting permission to receive this contact information to recruit participants for this study. We will only contact patients who have indicated an interest in hearing about research.

3) For Fairview patients that have elected to participate in University of Minnesota research, potential participants will be identified by Fairview staff from this repository, based on protocol eligibility criteria. Once identified, Fairview will mail the IRB-approved study recruitment letter to its patients on behalf of the research staff.

4) For physical flyers in the community, potential participants will self-identify based on limited information present. They will contact research staff via phone and/or email.

5) For internet and social media advertisements, potential participants will self-identify based on limited information present. They will contact research staff via phone and/or email or REDCap form.

6) For the patients of clinical groups affiliated with the University of Minnesota that are involved in the treatment of the target population potential participants will be identified by the clinician based on inclusion/exclusion criteria. The clinician will communicate information about the study to the patient with the opportunity to acquire more information from the research team. The clinician will provide contact information for the research team to the potential participant or will ask permission to provide the contact information of the potential participant to the research team if interested in participating.

7) Social media presence will direct traffic to IRB-approved website with contact information for the research team listed.

8) At community presentations potential participants will not be identified by research staff. Potential participants or parents of potential participants will have the opportunity to self-identify and request more information about research participation.

Supplement IV: Procedure for suicide and self-harm risk assessment

RAD Lab | University of Minnesota Department of Psychiatry and Behavioral Sciences

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Procedure for suicide and self-harm risk assessment

During the clinical assessment portions of this research study, research participants will be assessed for suicide risk and self-harm behavior with the assistance of a variety of research instruments (BDI-II, BSS, ISAS, CSSRS, KSADS, MINI, and SITBI), dependent on study specific protocol.

The following procedure outlines the assessment and management of suicide and self-harm behavior risk.

Suicidality: Interview and clinical assessments will include assessing the following suicide risk factors: ideation (duration, severity, and frequency), intent, plan, attempts, lethality, capability, preparatory acts, family/social support, current substance use and other factors impacting risk.

The presence of any above risk factors will then entail assessing the following: who else is aware of these risk factors (e.g., parents, mental health providers), what actions of prevention have been implemented and current subjective feelings of safety from the participant. From this information research staff will determine if any of the following action is needed: report/share information to/with others (e.g., participant's research team, family/parents of participant, participants medical or mental health team, child protective services/state

agencies), provide clinical referral to establish on-going care, or intervene in the case of an emergency.

Answers will be recorded and reviewed by RAD Lab investigators (Drs. Cullen, Klimes-Dougan or Reigstad) or research staff trained and supervised by RAD Lab investigators. Each clinical assessment will entail a RAD Lab investigator being on-call if not present at interview. As a result, a RAD Lab investigator (licensed psychologist or psychiatrist) will be consulted on the clinical case before safety action is taken. If a RAD Lab investigator is not available and safety concern exists, research staff will use their clinical judgment and take the action that best supports the health and safety of the research participant in that moment with the legal rights of this participant in the state of Minnesota in mind, while continuing to reach out to RAD lab investigator(s) for consultation until contact is made.

In the event of imminent risk, research staff will engage participants (and parents of minors) who indicate suicide risk in a conversation about suicidality and safety planning. This will entail addressing awareness of warning signs, eliminating access to lethal means, immediate access to emergency services (Emergency Department assessment, suicide hotline, txt4life), parameters of engaging emergency services, and establishing on-going clinical care. As indicated we will also involve the participant's treatment team. Research staff will strive to achieve an agreement with the participant and their parent(s) (for minors) on a safety plan that feels comfortable to all parties. Research staff will provide referral sources for clinical care, emergency contact information and encouragement for agreed upon safety plan.

Participants with severe suicidal ideation with intent, or those who cannot or will not agree to a safety plan will be referred for assessment in the Emergency Department (ED), for consideration of admission to the hospital. If this decision takes place during a visit at the Ambulatory Research Center (ARC) in the West Building of the Fairview Riverside Hospital or the Research in Adolescent Depression Lab at the University of Minnesota Medical School, the participant will be escorted or directed to the ED of the Fairview hospital, which is in close proximity of either locations. If the safety concern emerges during a visit at the Center for Magnetic Resonance Research or Center for Neurobehavioral Development we will either direct the parents to transport the patient to the ED and if possible call the ED in advance to inform them of the referral and transport to the ED, or will call 911. If the safety concern emerges following an at-home on-line visit or remote video conference visit, we will contact the participant and/or parent, engage in the safety conversation as discussed above, and as indicated, either direct parents to transport the participant to the ED or to call 911.

Self-harm behavior: Interview and clinical assessments will include assessing the following self-harm behaviors: type, frequency, severity, thoughts, family/social support and other factors impacting behavior.

In the event of significantly increased frequency or severity of self-harm behaviors and/or imminent concern of risk, research staff will engage participants (and parents of minors) in a safety conversation to discuss how to increase safety in the home, whether hospitalization is needed, and whether continuing in the study is appropriate. Research staff will also involve the participant's treatment team if it appears necessary for participant's safety or on-going

care. Research staff will provide referral sources for clinical care, emergency contact information and encouragement for agreed upon safety plan. Participants with self-harm requiring medical attention or those who cannot or will not agree to a safety plan will be referred for assessment in the ED as described above.