Antioxidant Therapy with N-acetylcysteine for Learning and Motor Behavior in Children with Neurofibromatosis Type 1

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Antioxidant therapy with N-acetylcysteine for motor behavior and/or learning in children with neurofibromatosis type 1

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ABSTRACT:
Children with neurofibromatosis type 1 (NF1) commonly suffer from the effects of cognitive, behavioral, and motor impairments. At present, there is no specific treatment for this NF1 complication. ***In this project, we will assess the safety and clinical benefit of N-acetylcysteine (NAC) as a pharmacological intervention in children with NF1***. This drug choice is based on the recent findings from mouse models to study the central nervous system manifestations of NF1 at Cincinnati Children's Hospital Medical Center (CCHMC). These findings revealed a role for myelin-forming oligodendrocytes in the control of nitric oxide synthases (NOS) and their product, nitric oxide (NO), in maintenance of brain structure and function, including regulation of behavior and motor control. Treating these mice with NAC corrected cellular and behavioral abnormalities. This data from animal models of NF1 along with uncontrolled clinical observations in children with NF1 suggest that the antioxidant compound, NAC, may reduce these impairments. Therefore, we propose performing a single center double-blind placebo controlled, prospective, Phase II study to explore safety, tolerability, and efficacy of NAC on motor behavior and/or learning in children with NF1 aged 8 through 16 years old. Participants will be carefully monitored for side effects. Primary and secondary outcome measures will be administered at baseline, follow-up, and post-treatment.

PURPOSE OF STUDY:
This is a phase II clinical trial with the goal to explore safety, tolerability, and efficacy of NAC on motor behavior in children with NF1 aged 8 through 16 years old. *We hypothesize that NAC therapy will improve motor function evaluated by the PANESS scale. This is based on studies demonstrating that NAC significantly improved impairments in the animal model of NF1. We will also analyze NAC effects on attention deficit and impulsivity in children with NF1.*

This study will also help develop novel predictive biomarkers of response to neurocognitive therapies in patients with NF1 which are needed to evaluate treatment outcomes.

We will gain information in children with NF1 about possible clinical benefit of anti-oxidant treatment and to develop and evaluate quantitative brain-based and blood biomarkers relating to presence of NF1, symptom severity, and response to antioxidant therapy. Clinically, 50% of children with NF1 are underperforming or failing at school [1]. This frequently leads to decreased educational attainment and fewer opportunities as adults. An important first step was our preliminary work using the PANESS scale and Transcranial Magnetic Stimulation (TMS)-evoked Short Interval Cortical Inhibition (rSICI) in children with NF1. We propose to develop and extend our understanding of NF1-related motor and learning behavior in response to antioxidant therapy with NAC. The purpose of the present study is to 1) evaluate tolerability, safety, and clinical benefit of NAC in this double-blind placebo controlled study using the motor function scale (PANESS); 2) to evaluate the effects of NAC on measures of NF1 neurocognitive symptomatology (ADHD/impulsive symptoms, executive function, working memory); and 3) to determine if TMS measurement (SICI) in children with NF1 will correlate with clinical effects of NAC treatment and evaluate utility of advanced brain imaging and spectroscopy measurements in children with NF1, and effects of NAC therapy.

We propose to study 58 children with NF1, ages 8-16 years, at baseline and after completion of 8 weeks of treatment with NAC, followed by a washout period of 4 weeks.

We believe this work has the potential to lay groundwork for future use of relevant biomarkers for treatment and outcomes research for NF1 as well as other biologically similar conditions, collectively designated the "RASopathies" (due to involvement of the RAS family of proteins) and ultimately to guide development of more effective treatments based on disease pathophysiology.

STUDY OBJECTIVE:

NAC Trial at Cincinnati Children's Hospital Medical Center (CCHMC)

We propose performing a single center randomized double-blind placebo controlled, prospective, Phase II study to explore safety, tolerability, and efficacy of NAC on motor behavior in children with NF1 aged 8 through 16 years old.

Hypothesis:

We hypothesize that NAC therapy will improve motor function evaluated by the PANESS scale. This is based on studies demonstrating that NAC significantly improved impairments in the animal model of NF1. We will also analyze NAC effects on attention deficit and impulsivity in children with NF1.

Specific Aim:

The primary outcome of this study is to characterize the effects of NAC treatment on motor function in children and adolescents with NF1 using the PANESS. We hypothesize that motor function scores rated with the PANESS scale will improve after treatment with NAC.

Secondary Aims:

1. To evaluate the effects of NAC on measures of NF1 neurocognitive symptomatology (ADHD/impulsive symptoms, executive function, working memory), we will use Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) committee recommended assessments tools DuPaul ADHD rating scale (ADHD-RS), Behavioral Rating Inventory of Executive Function second edition (BRIEF-2), Wechsler Intelligence Scale for Children, Fifth Edition (WISC-V) subtests, and Test of Variables of Attention (TOVA).
2. To determine if TMS measurement (SICI) in children with NF1 will correlate with clinical effects of NAC treatment.
3. To quantify microstructural properties of brain tissue based on water diffusion, glutathione GSH concentrations, and γ-aminobutyric acid (GABA) concentration using brain magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) in children with NF1. This will allow for regional correlation between imaging, spectroscopy and neuropsychometric outcomes. We will also determine if these magnetic resonance-based outcomes correlate with clinical effects of NAC treatment.
4. To evaluate safety and tolerability of NAC in children with NF1.
5. To explore motor function (PANESS) and brain-based measures (TMS, MRI-MRS, MRI-DTI) as biomarkers of impaired executive function (ADHD-RS; BRIEF-2; TOVA) in individuals with NF1.

BACKGROUND:

Neurofibromatosis type 1 (NF1)

Neurofibromatosis (NF1) is an autosomal dominant condition affecting many organs, resulting from mutations in the neurofibromin 1 gene [1]. Characteristically, individuals with this condition have cutaneous manifestations, most prominently hyperpigmented macules termed "café-au-lait" spots. The neurofibromin gene functions in part as a tumor suppressor, and persons with NF1 are susceptible to malignant and benign tumors in multiple organs. What is less well studied is that individuals with NF1 commonly suffer from difficulties with behavioral and emotional regulation including attention-deficit/hyperactivity disorder (ADHD) symptoms, learning disabilities, and developmental delays in motor function [2]. These cause substantial morbidity in childhood and throughout life. There are no effective treatments for motor behavior and/or learning difficulties for NF1 and other RASopathies. Lack of sensitive objective biomarkers that can evaluate motor system or behavioral symptoms causes difficulties in evaluating the efficacy of pharmacological interventions in NF1.

Preliminary results regarding motor function and brain-based measures as a rationale for additional study. A pre-planned analysis of predictor variables for NAC response has been performed, prior to breaking the blind in the randomized controlled treatment study. We performed these analyses on measures obtained at the baseline (pre-treatment) visit. These findings are now published, and some additional findings will be presented at scientific meetings. The strength of these preliminary findings warrants an expansion of the secondary aims, in order to validate these measures in a larger sample. Inclusion/exclusion criteria will be minimally changed to expand eligibility without compromising safety or study rigor. Individuals not wishing to participate in the NAC vs. Placebo treatment may elect to enroll and participate in an assessment of the baseline biomarkers. This will allow for additional assessment of the relationship between these measures and the NF1 diagnosis as well as for associations with motor and cognitive/behavioral impairments.

STUDY DESIGN:

This is a prospective single-center; randomized, double-blind, placebo-controlled Phase II study to determine the safety and efficacy of NAC on motor behavior and/or attention deficits of children with NF1 aged 8 to 16 years. Participants will be randomized to 8-weeks of treatment with NAC (70 mg/kg/day) versus placebo. This is a collaborative study between multiple specialties at CCHMC and external collaborators at Children's National Hospital and Technion Israel Institute of Technology. We have received IND approval from the FDA to use NAC for the application in this study (IND# 139468).

It is plausible and ethical to employ a placebo group, as no standard therapy with established efficacy is being withheld. There is no crossover in this study due to a lack of data concerning the length of possible washout effects. Primary and secondary outcome measures will be administered at baseline, after 8 weeks of treatment and at follow-up, 4 weeks after cessation of treatment to determine any washout effects.

In addition, the effect of NAC on measures of impulsivity, attention, executive function, and SICI and imaging biomarkers will be assessed. Participants will be carefully monitored for side effects by a medical safety monitor for the study. The safety of NAC will be evaluated using laboratory tests, clinical signs and adverse effects, which will be monitored at regular intervals over the 8-week treatment period.

An additional cohort of age/sex-matched NF1 participants will be recruited in order to characterize and validate biomarkers of brain function.

DURATION:

The current plan is for the study to last for 4 years. Data analysis and completion of study reports/papers will occur in the last 6 months of the study.

SELECTION AND RECRUITMENT OF PARTICIPANTS:

We plan to enroll 58 children ages 8-16 years with NF1 (**Figure 16**). Recruitment will be primarily from the NF1 and RASopathies Programs at CCHMC. The multidisciplinary program at CCHMC follows over 1500 individuals with NF1 and over 60% are under 20 years of age. We will also recruit from local events (including philanthropic and education events). Advertisements may also be posted on the NF1-associated websites including, but not limited to the Children's Tumor Foundation (CTF) website, NF1 facebook group, RASopathy facebook group, et cetera. Physical presence of research personnel in the clinic has been minimized in response to the covid-19 pandemic. Video recordings may aide recruitment and explanation of study assessments/details. These recordings may be distributed in a similar manner to other advertisements (NF1-associated websites, philanthropic/educational events, through interactions related to clinic visits, et cetera). Examples or video content would be a short description of TMS and PANESS procedures. A general outline of the study (risks/benefits, procedures, timeline, et cetera) as would be described during informed consent would also be advantageous.

An additional "Single visit, non-treatment" cohort will be recruited of 40 individuals with NF1 for a single "biomarker" study visit. These individuals will undergo motor function (PANESS) and brain-based measures (TMS, MRI-MRS, MRI-DTI) as biomarkers of impaired executive function (ADHD-RS; BRIEF-2; TOVA), but will not be assigned to receive NAC/Placebo.

We will use control data from age matched children ages 8-16 years, from previous studies (cohorts of 52 children with ADHD and 62 typically developing children).

We will also use advertising materials approved through the CCHMC Institutional Review Board (IRB). These include emails and small flyers which are posted at CCHMC locations. The study will be posted in clinicaltrials.gov.

Inclusion criteria ψ:

1. Male and females aged 8 - 16 years (up to 16 years and 6 months) at time of enrollment
2. Meets NIH diagnostic criteria for NF1
3. Abnormal PANESS study (score at or above the age/sex-based mean)
4. Participants must have a full-scale intelligence quotient (IQ) of 70 or above, as determined by neurocognitive testing within the last 3 years or during the enrollment process
5. Participants on stimulant or any other psychotropic medication should stay on a stable dose (no change in dose) for at least 30 days before entering the study. A stable dose should be maintained throughout the study until completion of all study visits.

Exclusion criteria:

1. Participants should not be receiving chemotherapy currently, or have received chemotherapy in the 6 months prior to entering the study

2. Active intracranial lesions (stable low-grade glioma is acceptable) ψ

3. History of seizure disorder or epilepsy. History of a single seizure that occurred more than 12 months prior to enrollment is acceptable. History of febrile seizures if the last febrile seizure occurred more than 12 months prior to enrollment is acceptable. Recurrent, unprovoked seizures (epilepsy) is sufficient for exclusion. ψ

4. Major Depression, Bipolar Disorder, Conduct Disorder, Adjustment Disorder, other major Anxiety Disorders, or other developmental psychiatric diagnoses, based on history

5. For females, pregnancy

6. Implanted brain stimulator, vagal nerve stimulator, ventriculoperitoneal shunt, cardiac pacemaker, or implanted medication port ψ

7. Asthma (bronchospasm has been reported as occurring infrequently and unpredictably when NAC is used as a mucolytic agent)

8. High risk of upper gastrointestinal hemorrhage. Examples: presence of esophageal varices or peptic ulcers

9. Current use of MEKINIST (MEK-inhibitor) or use within 30 days

ψ Indicates Inclusion/Exclusion Criteria for the treatment- and non-treatment cohort (no mark indicates exclusion requirements for the treatment-cohort only).

Inclusion of women and minorities: We will include males and females in numbers consistent with the distribution expected in the clinic populations. We will not exclude any participant based on race or ethnicity.

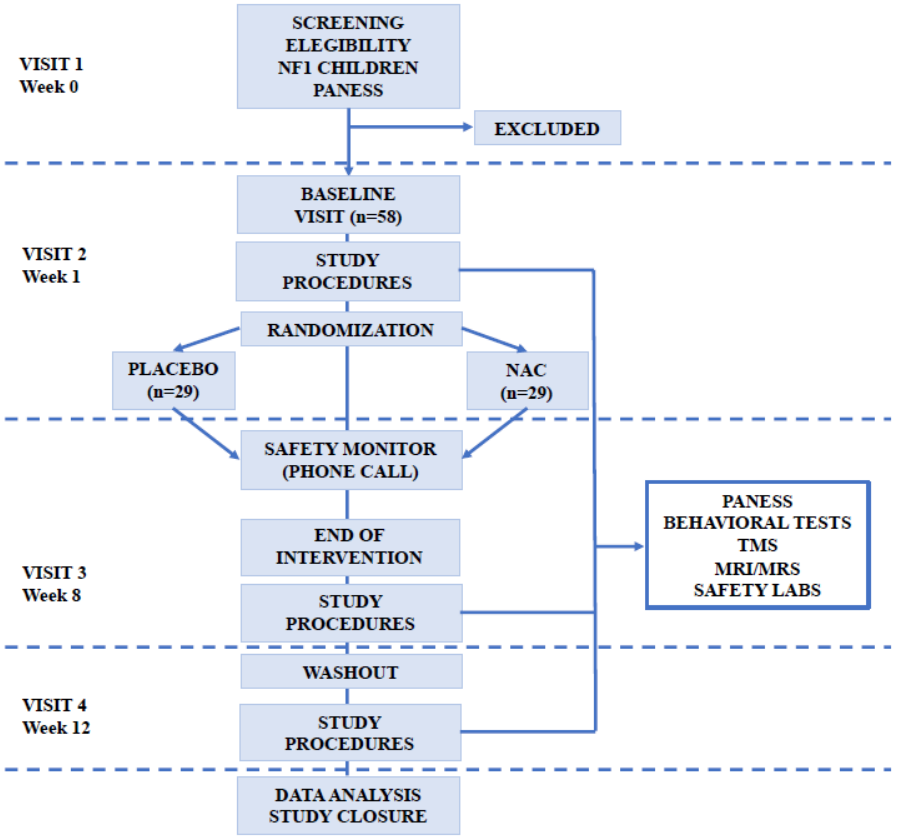

Figure 16: NAC phase II double-blind placebo controlled study design.

Randomization:

Following the screening period, each participant will be sequentially assigned to one of the 2 treatment groups: NAC or placebo. This randomization will be stratified to ensure balance of ADHD, ages and sexes between the intervention groups. Randomization will be implemented by the study statistician for this trial utilizing a permuted blocks approach. Password protection will be implemented to ensure that only certified clinical personnel are allowed to randomize patients and access the database. At the time of randomization, each participant will be assigned a unique medication kit identification number that will be used throughout the duration of the study.

Masking Procedure and Labeling:

In order to preserve the double-masking of the trial, only the investigation pharmacy will be unmasked. Both treatments (NAC and placebo) will be distributed from the investigational pharmacy. Except for the pharmacist, all staff will be blinded to the treatment sequence.

PROCESS OF OBTAINING INFORMED CONSENT:

The informed consent process will involve a member of the study team and the parent and child. If the individual is recruited over the telephone or by advertisements/mail/email, he or she will be sent a consent form and be contacted by a study coordinator to review the details of the study and answer questions. Interested families will then undergo screening as described in the *Eligibility Verification* document (a checklist of eligibility criteria).

Prior to study entry, all children recruited will undergo an initial screening lasting up to 15 to 20 minutes with a parent to determine their general suitability for inclusion or exclusion. This screening may be done over several days and can take place in person, on the phone, via email,

videoconference, and/or via electronic surveys such as REDCap. If determined to be eligible during the initial screen, the patient will enter the second phase of screening as outlined in the *Eligibility Verification* document.

After eligibility criteria are confirmed, the informed consent process will occur. Informed consent can take place in person, on the phone, via email, videoconference, and/or via electronic surveys such as REDCap. Following the consent process, eligibility screening will occur. The primary diagnostic screen will be the PANESS. Clinically administered PANESS scores performed within 90 days of enrollment can be used to confirm eligibility and as a baseline PANESS score for this study. Participants whom have not had a recent (within 90 days) PANESS assessment as a part of clinical care will be screened with PANESS. A set of behavioral and neuropsychometric assessments will be administered and families will be informed if they are eligible for the next phase of the study. The questionnaires are outlined in **Table 4**.

Parents/participants will deliver a teacher rating scale packet, with questionnaires/rating scales targeted to the classroom environment, to their child's teacher. Teachers will return completed rating scales to the study team via US mail using an addressed/stamped envelope provided by the study team. A letter will be included within the teacher packet iindicating that, by completing the questionnaires and mailing them in, they provide implicit consent. Communication with teachers and distribution of surveys may occur digitally via REDCap, telephone, videoconference, and/or email.

NAC/Placebo Treatment

After initial screening (visit 1) and baseline (visit 2, completed only on initial entry to the study) there will be approximately two on-site study visits (**Table 4**), involving approximately 120 minutes of diagnostic, motor, and neuropsychological assessment, a 90-minute TMS session, and 60 minutes of actual scan time (total = 5-6 hours). The administration of all interviews and cognitive testing will also be standardized with respect to personnel. A third day of testing may be added to accommodate the subjects' schedules and all research evaluations.

Non NAC/Placebo treatment cohort

These participants will undergo the baseline studies (TMS, MRI, motor testing, cognitive testing), as described in the prior paragraph, but no further on-site visits.

Consent

A separate consent form will be signed by each participant. Subjects 11 years and older will provide documented assent via a signature on the consent form.

Participation in this study will not change current medical treatment. Permission of the primary care/attending physician will not be sought.

To minimize the possibility of coercion, families will be encouraged to discuss this study in private (without the presence of study staff) and to ask the study staff questions to ensure understanding

of the study parameters. The decision to enroll can be considered for the duration of the enrollment period (e.g. until recruitment goal is reached).

For minor subjects who are brought to CCHMC by a person other than the parent or Legally Authorized Representative (LAR), the consent form may be sent to the parent for review. If so this consent process can take place over the phone, via email, videoconference, or REDCap eConsent. If the parent or LAR agrees to allow the participant to take part in the study, a copy of the signed consent will be returned and reviewed by a study coordinator before any research procedures occur. The consent form can be returned to CCHMC by fax, e-mail, regular mail, or REDCap eConsent response (which would be documented with a pdf in the participant's e-regulatory folder).

Electronic Consent (e-Consent) and Assessments:

To securely and efficiently maintain the data collected for this study, an electronic (eREG) binder will be utilized. As described below, paper regulatory documents will be scanned, verified, and filed in the eREG folder. To optimize the eREG process, electronic consent and assessments can be utilized. The format of electronic regulatory documents include pdf and/or REDCap. In addition to facilitating questionnaire distribution and document management, REDCap will enhance data analysis. Digital consent forms and questionnaires can be distributed to the participants/families who have reliable access to internet/email. Completion of electronic questionnaires and/or signing of e-Consent documents may take place during study visits using CCHMC resources (tablet, computer, signature pad) currently available to the study team.

Partial HIPAA Waiver

To facilitate eligibility screening of potential participants, the study team will implement a partial HIPAA waiver to screen, recruit, or determine eligibility of prospective subjects for a research study without screening-specific informed consent. As such, protected health information (PHI) access/retention for the purposes of identifying potential subjects is allowable. The use or disclosure of protected health information involves no more than a minimal risk to the privacy of individuals undergoing eligibility screening for this study. Eligibility screening for this study will not adversely affect the rights and welfare of the subjects. As with all research participants, security of protected health information (PHI) obtained during eligibility screening will be prioritized as described below.

PARTICIPANT WITHDRAWAL

A participant may withdraw from the study at any time. Participants considering withdrawal should contact the investigator so that the investigator can help you with a plan to stop the study drug safely.

The person in charge of the research study or the sponsor can remove participants from the research study without their approval. Possible reasons for removal include:

1. Adverse events requiring removal from protocol therapy
2. Inability or refusal to continue the study by patient/parent/guardian
3. Physician determines it is in patient's best interest

In the case of participant withdrawal/removal, data already collected may not be removed from the study database. The study team will request permission to collect data from your routine medical care. If the participant agrees, this data will be handled the same as research data.

The study team will inform the participant about any new information that may affect health, welfare, or choice to stay in the research.

STUDY PROCEDURES:

Study Variables and Measurements - Overview:
We propose to study all measurements at baseline, 8 weeks after treatment, and 4-week post-treatment (washout period) to assess for carry-over effects. We will compare PANESS and SICI measures in 58 children with NF1 to data already acquired at CCHMC for those 2 measures (**Figure 14)**. Next, these children will be treated with NAC (n=29) or placebo (n=29) as described above (**Figure 16).**

Treatment with NAC:
For this single center randomized double-blind placebo controlled, prospective, Phase II study, each subject will be dosed with approximately 70 mg/kg/day of NAC for 8 weeks. To facilitate drug compounding, three tiers of drug dose will be administered based on body weight as described in **Table 3**.

Table 3: NAC Dosing

Participant's weight (kg)	Dose (BID)
< 20	700 mg
20-40	1050 mg
> 40	1350 mg

*Max dose not to exceed 2700mg/day (1350mg BID)

We have treated 5 patients off-label with NAC at 900 mg twice daily with improved behaviors without side effects. NAC is a natural supplement that acts as an antioxidant and a glutamate modulating agent. It is widely available over the counter. We have received IND approval from the FDA to use NAC for the application in this study (IND# 139468).

To mitigate potential non-compliance, participants will be given a 'test dose' of the study placebo (capsule and/or powder) at the screening visit. If prescribed encapsulated drug, they will have to demonstrate that they can swallow the capsule. If prescribed powder, they will have to demonstrate that they can drink the powder without any issues.

Blood Acquisition and Storage:
Blood will be collected per institutional practice after participants are recruited and consented. No more than 3 ml of blood per kg of body weight will be collected during any single blood draw. No more than 5% of the patient's estimated blood volume will be taken for both clinically indicated and research purposes within a 24-hour time period. Samples will be logged and stored in the Cincinnati Children's Biobank.

Collection/use/disclosure of video recordings.

Certain study assessments (i.e.: PANESS) will be recorded using a tripod mounted video camera or similar device. The recorded assessment will be reviewed by multiple trained personnel to standardize grading across participants and improve the precision and accuracy of the scores. These records may be used for purposes of study, research, and teaching. If a clinically significant phenotype is noted in the recording that would benefit the scientific and clinical communities, portions of the recorded assessment may be published in scientific journals or presented at scientific conferences. The participant's/family's name will not be used. Information about the intention to record a portion of the study visits will be outlined in the consent form. Authorization is given when the consent form is signed by the participant/family.

Physical and Neurological Examination for Subtle Signs (PANESS) scale ψ:

At present, there are no clinical characteristics, neuropsychologic variables, phenotypic variants or biomarkers known to accurately predict treatment response of abnormal motor/learning behaviors in NF1 to NAC therapy.

Therefore, the primary outcome of the proposed study is motor function rated with the PANESS, a validated scale that consistently demonstrates significant impairments in children with ADHD, and which our preliminary data suggest may demonstrate more extreme problems in children with NF1.

The PANESS test measures timed movements, lateral preference, motor overflow, dysrhythmia, coordination, gait, balance, and motor persistence. The PANESS will be administered by staff, which has already been trained in PANESS administration. Our team has performed this examination as part of multiple studies. Administration of this measure is approximately 30 minutes.

When possible, the PANESS assessment will be recorded using a device such as a tri-pod mounted video camera. Digital files will be saved to a secure network drive to protect participant privacy. PANESS recordings can be reviewed by multiple personnel to improve precision and accuracy of the assessment and ensure reliability of the scores.

We will correlate the PANESS measurements with ADHD/behavioral symptoms using well established neuropsychological assessments. Thus, we will assess ADHD attention and hyperactive/impulsive symptoms with the DuPaul, BRIEF-2, and TOVA clinical rating tests. Parent symptom ratings are precise and appear to be quite sensitive to change and capture real-world functional change.

Psychometric Measures

All primary measures will first be administered at screening. ***We will use the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) Neurocognitive committee reviewed and recommended assessment tools to assess symptomatology in NF1***. The length of time required for the child to complete the baseline neuropsychological assessment is estimated to be approximately 90 minutes, including self-report questionnaires. Parent questionnaires can be completed simultaneously while the child is being evaluated and can be completed in about 30

minutes. All neuropsychological assessments that will be used in this study are published tests that have normative data and established reliability and validity for the age range in this study. These tests are all extensively used in clinical and/or research settings. All tests have standardized instructions for administration ensuring there will be no site variation in administration.

To minimize any test-retest effects for neuropsychological outcome measures, alternate forms will be used where possible, to minimize potential practice effects. Furthermore, the use of a placebo group will allow us to identify any potential practice effects and distinguish whether any changes in the treatment group are larger than those accounted by practice effects. In addition, statistical analysis of the data will be conducted to account for confounding variables such as practice effects.

The WISC-V testing is optional for individuals who will not be receiving NAC/Placebo. However, the PANESS and the neuropsychometric testing below will be performed in all "single-visit, non-treatment" participants.

DuPaul ADHD-Rating Scale,(ADHD-RS) Home (parent) form ψ

This 18 question scale is based on the DSM-5 diagnostic criteria and is widely used in clinical trials and routine care. ***The ADHD-RS rated high in the REiNS committee for assessment of ADHD symptomatology in NF1.*** Administration of this measure is approximately 15 minutes. The ADHD-RS has high internal consistency (alpha>0.90) and good test-retest reliability (r=0.85). The rating scale has excellent convergent and discriminant validity.

Behavioral Rating Inventory of Executive Function Second Edition (BRIEF-2) ψ

The Behavioral Rating Inventory of Executive Function (BRIEF-2) is a parent-reported symptom rating form that is used regularly in NF1 research and is recommended by the REiNS Neurocognitive Committee for the assessment of executive functions in NF clinical trials. This assessment measures executive function behaviors at home and at school for children and adolescents (age 5–18). This scale would give a sense of if any changes are occurring in real-world executive function. The Parent Form is completed by the child's parent or guardian. It contains 86 items, with eight clinical scales and two validity scales. Behavior descriptors of a child are rated on a three-point Likert scale: "never," "sometimes," "always." Items were developed to capture everyday behaviors associated with executive functions, and tap eight domains including: initiate, inhibit, shift, plan, organize, self-monitor, emotional control, and working memory. These scales are combined to form two broader indices (Behavioral Regulation and Metacognition) and a Global Executive Composite score. Administration of this measure is approximately 15 minutes. The BRIEF-2 has high internal consistency, with a reliability coefficient of 0.90, and good test-retest reliability (r=0.82). This measure has well established convergent validity with other measures of behavior and intelligence, and good discriminant validity has been demonstrated against measures of emotional and behavioral functioning.

Test of Variables of Attention (TOVA) ψ

The TOVA is a computerized, objective measure of attention and inhibitory control normed by gender for ages 4 to 80+. The test is a 22-minute test administered to the participant that provides objective data on response time, inattention, and impulsivity. Test-retest reliability of the TOVA is good, ranging from .74 - .87 with a 1-week interval [66]. The TOVA has also demonstrated good convergent and discriminant validity.

Wechsler Intelligence Scale for Children, Fifth Edition (WISC-V) (ψ optional)

Working Memory Index. This index of the WISC-V consists of two scales – Digit Span and Picture Span, which assess auditory and visual working memory in school-aged children. ***This objective measure of working memory has been recommended by the REiNS Neurocognitive Committee (specifically Digit Span) as an outcome measure for working memory in NF clinical trials.*** This is a standardized assessment tool used very frequently in clinical care and clinical research. Administration takes approximately 10 minutes and produces age-referenced standardized scores for each subtest as well as the Index score.

Processing Speed Index. This index of the WISC-V consists of two scales – Coding and Symbol Search, which assess processing and response speed in school-aged children. This is a standardized assessment tool used very frequently in clinical care and clinical research. Administration takes approximately 10 minutes and produces age-referenced standardized scores for each subtest as well as the Index score.

The WISC-V has high internal consistency, with reliability coefficients for subtests, primary index scores, and FSIQ ranging from 0.80 to 0.96. It also has good test-retest reliability, with coefficients ranging from 0.80 to 0.94 on subtests, primary indexes, and FSIQ. The WISC-V has high criterion validity and good convergent and discriminant validity.

We will provide a referral for clinical evaluation to potential participants that receive an ineligible IQ rating (<70)

Cognitive Function: Kaufman Brief Intelligence Test, Second Edition (K-BIT-2) ψ

Each child will be administered a psychological screening battery by trained researchers under the supervision of a licensed psychologist, to assist in evaluation of inclusion and exclusion criteria, and to describe the sample. Within the constraints of limited time and resources, we have selected the following test to evaluate cognitive functioning and learning problems. Participants will undergo a brief IQ screener at baseline using the Kaufman Brief Intelligence Test-2 (KBIT2). The K-BIT-2 has high internal consistency (a ranging from 0.89 to 0.96) and composite test-retest reliability (r=0.90). The measure also has moderate to high correlations for construct and concurrent validity.

We will provide a referral for clinical evaluation to potential participants that have incidental findings noted in the MRI/MRS

The Imaging Research Core (IRC) at Cincinnati Children's Hospital will assign a radiologist to review MRI/MRS data and create a report to outline any incidental findings. The incidental finding report is essentially a safety report where the radiologist documents if there are abnormalities separate from their underlying diagnosis. Upon notification of incidental findings, the study team will review the report within 3 business days. The study team will determine whether a clinical referral and/or correspondence with the family is necessary. If the study team determines that the family should be notified (with or without the need for a clinical referral), a member of the study team will contact the family to schedule a call with the study doctor or designee.

At the study doctor's discretion, we may provide reports or referrals for clinical evaluation to families regarding anxiety and depression assessments

Pediatric Anxiety Rating Scale (PARS) [21]

The PARS is a clinician rated scale that is completed following interview with the parent and child. The PARS has two sections, a symptom checklist and severity items, each of which is linked to the past week. We chose PARS over several possible self-report scales because young children may not be able to complete a self-report scale. Administration of this measure is approximately 10 minutes. The PARS has adequate test-retest reliability (ICC=0.55) and fair internal consistency (a=0.64). This scale also has adequate convergent and divergent validity.

Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS)

The KSADS is compatible with the DSM-5 and has been validated in multiple research and treatment settings. The KSADS cross-cutting measure (DSM-5 CC-SM) is an abbreviated version of the extended form. The abbreviated measure is a reliable way to recognize mood, anxiety, psychotic, and disruptive behavior disorders and is a useful screen for eligibility of potential participants. The DSM-5 CC-SM are designed to be self-report measures, completed by the parent and child. The assessment typically is completed in 10 minutes.

Children's Depression Rating Scale (CDRS) [22]

The CDRS is a clinician-completed scale that rates presence and severity of depressive symptoms, based on interviews with the parent and child separately. There are 17 behavior items of affective state, rated dimensionally normed for children 6-12 years. Administration of this measure is approximately 10 minutes. The CDRS has good test-retest reliability (a=0.86). The scale has a good validity correlation (0.87) and high convergent validity.

Suicide assessment:

Per FDA guidance we will screen for Suicidal Ideation and Behavior at each visit. The Columbia Suicide Severity Rating Scale (C-SSRS) is a fast assessment of current and past suicide ideation that is used throughout Cincinnati Children's Hospital. The C-SSRS is administered by a trained researcher and takes approximately 5 minutes to complete.

Review of anxiety, depression, and C-SSRS responses and referral for clinical evaluation:

Study doctor (or designee) will verbally administer and then review each suicide assessment. The 'Columbia-Suicide Severity Rating Scale Screen with Triage Points' document outlines the planned course of action based on the participant's response. In particular, if a participant/family discloses any suicide ideation at any point in this study, the study staff will contact the participant's mental health provider or primary care provider and/or *Psychiatric Intake Response Center (PIRC)* at CCHMC for further assessment and guidance within 24 hours.

> *The Pediatric Intake Response Center (PIRC) manages psychiatric patient consultation and intake for Cincinnati Children's Hospital Medical Center. Staff members are available 24 hours a day, seven days a week at 513-636-4124 or psychiatryresponse@cchmc.org .*

Rationale for Imaging and Neurophysiology Studies – Overview:
As an additional secondary endpoint, we will use **TMS and DTI**, because quantitative biomarkers of white matter structures may help measure and predict behavior in NF1 and facilitate interventions into clinical practice. **DTI**, including at least one of its derived parameters, **FA,** shows global changes in NF1 brains which are consistent with white matter dysfunction [11]. In other brain disorders, DTI indices have shown sensitivity to changes after treatment [67-69]. It is clear that white matter properties can also be evaluated using neurophysiological techniques, including Transcranial Magnetic Stimulation. TMS studies are generally brief (30 to 60 minutes), inexpensive, readily repeatable, and do not require sedation. TMS may be helpful for identifying clinically useful, specific brain biomarkers and assessments related to motor and learning difficulties in NF1 and, ultimately, other conditions involving RAS pathways. Specifically, we will test motor system inhibitory physiology (**SICI**), measured using TMS. Preliminary measures in our NF1 population show abnormalities similar to established findings in ADHD obtained in our TMS laboratory at CCHMC.

Transcranial Magnetic Stimulation (TMS) ψ:
Paired pulse TMS will be performed using a Magstim 200^{R} stimulator (Magstim Co., New York, NY, USA) connected through a Bistim^{R} module to a 90 mm circular coil. The coil is placed with its center near the vertex in the optimal position and orientation for producing a motor evoked potential (MEP) in the right first dorsal interosseous (FDI) muscle. The electromyogram (EMG) is recorded from the right (or dominant) FDI with surface electrodes, amplified, and filtered (100/1000 Hz) (Coulbourn Instruments, Allentown, PA) before being digitized at 2 kHz and stored for analysis using SignalR software and a Micro1401 interface (Cambridge Electronic Design, Cambridge, UK).

The resting MEP threshold (RMT) is measured using a method similar to that described elsewhere [70] by setting the stimulator to 20 % of maximum output and increasing by 10 % increments until a MEP is obtained. The intensity is then decreased in increments of 1 % of maximum stimulator output until an intensity is reached where 10 averaged stimuli failed to produce a MEP. Active MEP threshold (AMT) is determined by having the participant moderately contract the abductor pollicis brevis with auditory feedback to maintain a constant level of EMG. The stimulator output is decreased in increments of 1 % of maximum output from the RMT until 10 averaged stimuli fail to show a MEP above background.

SICI inhibition is measured in resting muscle with a paired-pulse paradigm using paired vs. single pulse conditions[71]. The first condition is a single test pulse, delivered at an intensity that consistently produces a 500- 1500 mV MEP. In the second condition, a subthreshold stimulus (60% of RMT) is followed after by a test pulse. Twenty trials are performed for the single-pulse condition, and 20 each for the other interstimulus intervals 3 milliseconds (ms), 10 ms. Relative to the single pulse response, the 3 ms interval is inhibitory (SICI) and the 10 ms interval is excitatory (intracortical facilitation). The order of the intervals is varied randomly, and the interval between trials varies randomly by <20% around a mean of 6 seconds. SICI is expressed as a ratio of the mean MEP amplitude after the conditioned pulses divided by the mean MEP amplitude after the single pulses. A detailed review of systems will be obtained before and after TMS, and participants will be provided the number of the investigator and study coordinator to call if there are concerns about possible side effects after the laboratory visit.

Magnetic Resonance Imaging (MRI) ψ:
The MRI protocol will be comprised of un-sedated imaging sequences providing high-resolution detail of brain tissue structure with a primary focus on diffusion FA. A three-dimensional T1-weighted volume will be acquired with 1 mm isotropic resolution and strong contrast between gray matter and white matter tissue. This image volume will be used for anatomic reference and may also serve to explore voxel-level volumetric impact of NF1. A DTI sequence (≤ 2 mm isotropic resolution) will follow, which is directionally sensitive to the rate of water diffusion in brain tissue. This sequence will be designed to measure diffusion rates in 32 or more directions for two or more levels of diffusion weighting (e.g., b = 1000 s/mm^2 and 2000 s/mm^2) to allow voxel-wise determination of not only FA, our primary DTI marker, but also additional microstructural indices that may be sensitive to NF1 and changes with NAC [72, 73]. The DTI data are unique in this study's context in that they are brain-wide: this assessment will be done per voxel, or, alternatively, per predefined white matter Region of Interest (ROI). These additional indices, as well as the potential for regional volumetric assessments, will be exploratory and allow us to address contingencies. DTI will be especially focused on white matter, where restricted diffusion of axonal structures leads to a high degree of anisotropy that reflects tissue integrity.

The T1-weighted image will be processed using one of the commonly available software packages for MRI. Processing will include segmentation into gray matter, white matter, and cerebrospinal fluid tissue types and normalized by transformation into a common spatial template [74]. DTI volumes will have been aligned to each other and with the T1 anatomic image and normalized to the same template space before processing in software to extract FA on a voxel-wise basis. In healthy white matter, diffusion will be greatest along axonal fibers and FA describes the degree to which axial diffusion exceeds transverse diffusion. Besides FA, this processing will provide estimates of mean diffusivity (MD) and the radial (RD) and axial (AD) diffusivities, which have shown changes with NF1 [11, 75, 76] and its treatment [54]. ***Increased FA with decreased RD might suggest improvement in myelin.*** Images may be processed in other ways to extract additional structural information. The T1 segmentations may be analyzed as tissue probability maps for voxel-based morphometry [77]. The DTI data may allow axonal fiber tractography to ascertain structural connectivity between brain regions pertinent to NF1, including tracts of the cingulum and corpus callosum. *Our primary imaging outcomes in white matter will be voxel-wise comparison of FA between NAC and Placebo groups and regional correlation between FA and neuropsychometric outcomes pre and post-NAC*. We have healthy reference DTI (FA) data for children 8 to 16 years for comparison to NF1. Since all images will have been normalized to the same space, voxel-wise comparisons of indices across subjects will be possible.

Magnetic Resonance Spectroscopy (MRS) ψ:
We will use MRS to quantify glutathione (GSH) and GABA levels in children with NF1 to assess effects of NAC therapy. This will allow for regional correlation between MRS and neuropsychometric outcomes. The HERMES MRS sequence (echo time 68 ms, repetition time 2000 ms) requires 11 minutes per 27 mL location of interest sampled in the brain. MRS is performed within the medial frontal cortex including dACC [78, 79]. MRS detects neurochemical concentrations within key neural elements (neurons, axons, glia) reflecting structural composition such as N-acetyl aspartate, creatine with phosphocreatine, total choline, and myo-inositol and functioning with glutamate, GSH and GABA. The HERMES method provides GSH, GABA and

GLX; the GABA-off and GSH-off portion of the HERMES experiment provides a spectrum for standard metabolites. MRS data are postprocessed with LCModel and Gannet software and adjusted for tissue composition and neurochemical relaxation, to produce concentrations.

Table 4: Schedule of Assessments

	Visit 1	**Visit 2**				**Visit 3**		**Visit 4**
	Screening	**Baseline**	**Abbreviated Remote monitor and safety**	**Remote monitor and safety**	**Abbreviated Remote monitor and safety**	**End Intervention**	**Remote monitor and safety**	**Follow Up**
Task	**Week 0**	**Week 1**	**Week 2**	**Week 4**	**Week 6**	**Week 8**	**Week 10**	**Week 12**
Review Eligibility Criteria ψ	X							
KBIT-2 (Kaufman Breif Intelligence Test, Second Edition) ψ	X							
KSADS (MedHis)	X							
Cognitive Function Screening ψ	X							
Test dose	X							
Informed Consent ψ	X							
Brain Imaging (MRI/MRS) ψ		X				X		X
Urine Pregnancy (Females only)	X	X						
Vitals*		X				X		X
Safety labs*		X				X		X
Physical Exam*		X				X		X
ADHD-RS (DuPaul) ψ		X		X		X	X	X
KSADS (CC)		X		X		X	X	X
PARS (Pediatric anxiety rating scale)		X		X		X	X	X
CDRS (Children's depression rating scale)	X	X		X		X	X	X
C-SSRS (Columbia Suicide Severity Rating Scale)	X	X		X		X	X	X
Wechsler Intelligence Scale for Children, Fifth Edition (WISC-V) ψ	X	X				X		X
Behavioral Rating Inventory of Executive Function (BRIEF-2) ψ		X				X		X
Test of Variables of Attention (TOVA) ψ		X				X		X
PANESS scale ψ		X				X		X
TMS assessment ψ		X				X		X
NAC/Placebo Prescription		X						
Med Log		X	X	X	X	X	X	X
AE assessment (MedDRA)		X	X	X	X	X	X	X
Follow-up plan								X

* Indicates clinical procedures performed as standard of care
ψ Indicates research procedures to be completed in both treatment and non-treatment cohorts

DATA ANALYSIS/METHODS:
NAC Trial at CCHMC

We propose performing a single center randomized double-blind placebo controlled, prospective, Phase II study to explore safety, tolerability, and efficacy of NAC on motor behavior and/or attention deficit in children with NF1 aged 8 through 16 years old.

Determination of sample size:
No data exist on the effects of NAC in children with NF1. Clinical improvement will be evaluated as a change score ($PANESS_{post\text{-}NAC} - PANESS_{baseline}$). Those subjects whose severity PANESS score decreased by at least 20% from baseline to week 8 will be considered clinically meaningful. The sample size calculation was based on a two-sided t-test to detect the difference in change scores between two groups. We assume the PANESS score does not change for patients in placebo group. A sample size of 26 participants per group (52 in total) achieves 81% power to detect an effect size of 0.8 with significance level of 0.05 [80]. To allow for a 10% dropout rate prior to the 12-week point, a total of 58 participants will be enrolled.

Randomization:
Following screening period, each participant will be sequentially assigned to one of the 2 treatment groups: NAC or placebo. This randomization will be stratified to ensure balance of sexes and ADHD between the intervention groups. Randomization will be implemented by the study statistician for this trial utilizing a permuted blocks approach. Password protection will be implemented to ensure that only certified clinical personnel are allowed to randomize patients and access the database. At the time of randomization, each participant will be assigned a unique medication kit identification number that will be used throughout the duration of the study.

Statistical analysis:
All analysis will be performed with SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Spearman's correlation coefficient will be used to evaluate the univariate association between primary outcome (improvement of PANESS scores) and other neurophysiology and brain imaging measurements. Multivariate linear regression model will be used to assess the correlation between the outcomes and the risk factors adjusting for all possible confounders. Model selection criterions such as stepwise method will be employed when necessary. A p-value less than 0.05 will be considered statistically significant.

1. We will assess behavior vs. neurophysiology correlations:
   1) two-month PANESS change ($PANESS_{post\text{-}NAC} - PANESS_{baseline}$) vs. SICI change ($SICI_{post\text{-}NAC} - SICI_{baseline}$).
   2) two-month DuPaul change ($DuPaul_{post\text{-}NAC} - DuPaul_{baseline}$) vs. SICI change ($SICI_{post\text{-}NAC} - SICI_{baseline}$).
   3) two-month BRIEF change ($BRIEF_{post\text{-}NAC} - BRIEF_{baseline}$) vs. SICI change ($SICI_{post\text{-}NAC} - SICI_{baseline}$). Paired t test comparisons of single pulse TMS responses (resulting from pTMS) will be performed to test whether, at each session, single pulse TMS responses differed. The mean single pulse TMS responses for each individual constitute the denominators for the SICI and ICF ratios and need to remain consistent for changes in SICI to be interpretable.
2. We will assess primary behavior (PANESS) vs. neuroimaging correlations:
   1) two-month PANESS change ($PANESS_{post\text{-}NAC} - PANESS_{baseline}$) vs. Fraction Anisotropy (FA) change ($FA_{post\text{-}NAC} - FA_{baseline}$).

2) twomonth PANESS change ($PANESS_{post\text{-}NAC} - PANESS_{baseline}$) vs. MRS GSH change ($GSH_{post\text{-}NAC} - GSH_{baseline}$).
3) two-month PANESS change ($PANESS_{post\text{-}NAC} - PANESS_{baseline}$) vs. MRS GABA change ($GABA_{post\text{-}NAC} - GABA_{baseline}$). The current HERMES method data analysis of MRS can observe GSH concentration changes in the brain of over 20%. We will determine if NAC dose changes GSH in brain and correlates with the primary outcome of study (PANESS).

For analyses of change over time, mixed models which include subject-specific random effects will be used to account for the correlation of multiple measurements on each subject and capture between-subject variation in rates of change. Unless otherwise noted, the Type I error rate for hypothesis tests and confidence intervals will be controlled at a=0.05. To assist interpretation, the size of effects of interest in each model will be represented by R2 (proportion of outcome variation explained by predictors) for linear regression models and/or Cohen's *d* (difference in group means, in standard deviation units) for two sample t-tests [81].

Exploratory and descriptive data analysis will precede all inferential analyses to ensure that distributional assumptions are satisfied. In addition to computation of basic descriptive sample statistics (measures of central tendency, variability, and bivariate association), graphical tools such as histograms and scatterplots will be used to assess, e.g., approximate normality, linearity, and presence of influential outlying observations. If substantial violations of parametric assumptions are detected, we will implement non-parametric alternatives, including classical rank-based procedures as well as computationally-intensive sample re-use procedures such as the nonparametric bootstrap. Non-parametric procedures generally have less power than parametric procedures when the assumptions of the latter are met, but often have markedly better properties when the assumptions fail.

In the event that a participant is lost to follow-up, an incomplete series of observations can still be analyzed using the mixed-model methodology, provided that the dropout can be assumed to be missing at random in the sense that the probability that data are missing depends only on observed covariates (e.g., prior values of the outcomes, treatment assignment), rather than the unobserved values themselves. This assumption cannot be directly verified from the data. When it holds, however, mixed-model procedures yield the most efficient unbiased estimates, and implicitly impute the missing data using a subject-specific linear trend estimated from the non-missing observations. This effectively assumes that observed trends would have continued, in contrast to the "last observation carried forward" assumption that future observation would remain at a constant level equal to the last value. Neither assumption can be empirically proven correct. As a sensitivity analysis, therefore, the last available observation will be substituted for the protocol-defined last observation, provided that the participant had received at least one dose of study medication prior to the last observation. It is possible to determine whether participants who drop out are systematically different from those who do not with respect to observed data (e.g., baseline values, treatment assignment, demographic and clinical covariates). Logistic regression analyses will be used to explore whether such variables are predictive of dropout.

FACILITIES and PERFORMANCE SITES:

Cincinnati Children's Hospital Medical Center

1) Division of Human Genetics

2) Division of Neurology; TMS laboratory
3) Imaging Research Center

External Collaborators (data analysis):
1) Neuropsychology, Children's National Hospital
2) Neuroscience, Technion – Israel Institute of Technology

POTENTIAL BENEFITS:

The purpose of this study is to evaluate whether participants with NF1 will benefit from improved thinking/learning, behavior, and movement in response to eight (8) weeks of treatment with N-Acetylcysteine (NAC). There is a potential for direct benefit by participating in this study if the treatment improves the symptoms of motor behaviors and learning associated with NF1. However, there is a possibility of no direct benefit to the participant.

In addition to possible benefits of the study drug, medical records and/or study assessments will be reviewed by our multidisciplinary team that includes geneticists, neurologists, neuropsychologists, and radiologists. Any findings that may possibly impact the participant's health and well-being will be reviewed with the parent/caregiver and appropriate referrals for further testing or management will be made by a physician on the study team. Returned results will be based solely on the baseline assessment, when the most complete information is available, and when any practice effects have not yet occurred that could distort norm-referenced interpretations. This will be of use to help tailor other interventions to individual participants.

When we finish the research, we expect that we will know more about NF1 and the associated cognitive (thinking) and motor (movement) concerns. This may help participants in this study as well as other children with NF1.

POTENTIAL RISKS, DISCOMFORTS, INCONVENIENCES AND PRECAUTIONS:

Known and potential discomforts or hazards of single and paired pulse TMS:

Single and paired pulse TMS has been used at CCHMC under Dr. Gilbert's direction since 2001 for research only. Potential discomforts are mild and transient. In a prior study of 40 healthy and ADHD children, Garvey et al asked children to rank TMS compared to other childhood activities. TMS was ranked preferable to 1) a "shot"; 2) going to the dentist; and 3) a long car ride. The following mild, transient effects were reported in our prior study of 35 children and adults: scalp discomfort (12%), hand weakness (9%), headache, neck pain, arm pain, and arm tingling (6%), hand pain, decreased hand dexterity, hearing changes, and tiredness (3%). All of these had resolved by the following day. There were no physical findings after TMS supporting the subjective descriptions of loss of strength or dexterity. A prior common concern about use of TMS was the risk of seizures [28]. We follow recommended guidelines [28] and have seen no seizures in children or adults studied at our center. In addition, more recent studies even in children with epilepsy suggest that the risk of TMS inducing seizures is extremely low.

Known and potential discomforts or hazards of MRI/MRS:

There are no known risks injury associated with a brain MRI/MRS. A few common less serious risks have been reported and are described here. Participants susceptible to claustrophobia may experience anxiety during the scan. The noise generated by the MRI/MRS machine can be

uncomfortable for some participants. Ear protection will be available to minimize the noise exposure. Participants may experience mild muscle/nerve stimulation that may feel like a twitching sensation.

Participants with a medical implant or external accessory device may experience complications with that device while undergoing an MRI/MRS. One potential complication is that the device may move in response to the magnet. Implants, external and accessory devices may heat up in response to the magnet, which could lead to burns. A third potential complication is the potential that electrochromic implants, external and accessory devices may malfunction in response to the magnet. To mitigate the risk of harm, we have included the use of such implants/devices as an exclusion factor for this study.

Known and potential discomforts or hazards of NAC
NAC is FDA approved for treatment of acetaminophen overdose, respiratory disease due to mucous obstruction in acute and chronic settings in children and adolescent. Common side effects include pruritus, rash, urticaria, diarrhea, nausea, and vomiting. NAC may not be effective for every participant, therefore participants may experience a continuance or worsening of their condition, including school performance or social interaction, while in the study.

Precautions, risk minimization:
The protocols include a number of precautions to minimize risk. Standard of care will be followed for all procedures performed to decrease the risk of infection, pain, bleeding, and bruising. The TMS laboratory was established in 2001 in consultation with Dr. Eric Wassermann, an internationally known researcher in transcranial magnetic stimulation. A trained and TMS-administration certified neurologist will be present during TMS. All subjects will wear 34 NRR earplugs or headphones during TMS if the head is stimulated at an intensity of over 90%. Standard exclusion criteria are applied for participation in this study. Detailed questioning for any adverse events will occur after the experiment and the next day after the study. Access to the Magstim Stimulator is Limited – these TMS devices are kept in room 4 of the EEG lab on the seventh floor of the A tower. The laboratory is continually monitored by CCHMC during business hours and is locked at all other times.

The effects the study drug and procedures would have on a fetus are unknown. When deemed appropriate by the study doctor or designee, pregnancy testing will be utilized before study procedures will take place. Females that test positive for pregnancy will be excluded or withdrawn from the study. Risks to a fetus conceived while on study drug will be reviewed with all parents. A conversation about risks and the importance of contraception while in this study will also be conducted with participants when deemed appropriate by the study doctor or designee. Consent to participate in this study includes promise of compliance with appropriate contraceptive measures. Females will use an effective means of birth control (i.e. abstinence, hormonal, or intrauterine device) during the study and for 30 days after taking the last dose of study drug. Males will use an effective means of birth control (i.e. abstinence or double-barrier contraception) during the study and for 90 days (3 months) after taking the last dose of study drug.

The method of monitoring study conduct:

Adverse events will be reviewed using direct questions with a detailed review of systems on the day of the study. The principal investigator or designee is responsible for reporting adverse events to Cincinnati Children's Hospital Medical Center Institutional Review Board. Unanticipated events will be reported to both IRBs, according to the individual IRBs' requirements.

DoD representatives can independently review and inspect research associated with this study. They can prohibit research that is determined to present unacceptable hazards or is non-compliant with DoD regulatory requirements.

Methods for maintaining data quality and confidentiality:
Data are maintained in case report folders identified only by an anonymous subject ID. They will be kept in a secured area of Cincinnati Children's Hospital. All data kept in computerized files are in computers or a server with restricted, password-protected access. Only the primary investigator and designated study staff within his laboratory have access to case report folders and computer passwords (restricted by access level). A participant will be identified throughout the central database by his or her unique subject identification number (SID). Information which could identify a subject, such as name, address or social security number, will either not be stored electronically. A database audit trail of all data element changes over the life of the study will be maintained.

Data will be kept in a separate data base without demographic and clinical information and will be merged during the analytic stage of the study. Data entry, verification and validation will be carried out using our proprietary clinical data management system. We have a fire-wall-protected and local-network-based database management system for paper-based data collection, reports processing and information handling.

The study PI is responsible for overseeing execution of the research. Oversight responsibilities apply to all human subjects' research, whether or not it is determined to be exempt from the regulations.

RISK/BENEFIT ANALYSIS:

In the opinion of the investigative team, this study, based on our experience with NAC and with administering TMS as well as the published literature on NAC and TMS, involves minimal risk, with potential for direct benefit.

DATA SAFETY AND MONITORING:

Data Safety Monitoring Plan:
Steps to be taken to assure the accuracy of the study data: The study team will review case report forms, source data, and spreadsheet entries once annually to ensure accuracy of data entry.

Adverse events and unanticipated events will be classified based on severity and attributed to individual components of study participation. For example, if a subject reports nausea at the end of the TMS baseline visit, this will be classified based on severity rating from the subject (mild, moderate, severe in the case report forms) and relationship to study tasks will be ascertained as well as possible (related to TMS, related to study medication, related to both, related to neither, unsure) on a case report form.

An external safety monitor will not be utilized for this minimal risk study. The study is deemed minimal risk because of the unrestricted availability of the study drug.

PRIVACY AND CONFIDENTIALITY:

Privacy of the individuals participating in this proposed study will be maintained through non-identifying subject ID codes (SID), locked storage and password protected files. External collaborators will have access to de-identified research records only. Distribution of participant medical records to external collaborators will be done via secure email transmissions. The study log will be maintained in a password protected folder on the desktop computer of the principal investigator. Case reports and spreadsheets will refer to subjects by number only. Subjects will be informed that, if necessary, the IRB or FDA may review the data.

DoD representatives will be allowed to independently review and inspect the awardee's research. This may include access to identifiable information or protected health information. Participants will be informed about this risk to confidentiality.

COST OF PARTICIPATION:

There will be no charges to participants or to third party payers related to study visits or procedures in this study. NAC is an FDA approved medication. NAC will be provided for the 8 week duration of the study to all participants.

COMPENSATION/PAYMENT FOR PARTICIPATION:

Families will be compensated for time, effort, and travel as described in the table below. For the first 3 study visits, each participant will receive $25. Study visit 4 has additional conditions (i.e.: return of drug diary and unused drug), therefore, each participant will receive $100 for that visit. Total compensation for this study per participant is $175. Reimbursement will be distributed within 90 days of study completion or withdrawal.

Payment Schedule			
Visit 1 ψ	Visit 2	Visit 3	Visit 4
$25	$25	$25	$100

ψ Indicates treatment- and non-treatment cohort payment

COMPLIANCE AND SAFETY MONITORING:

Monitoring and Managing Adherence to Treatment:

Pill/Sachet counts and/or review of the medication log will be used to estimate compliance, along with queries about missed doses. We will require a standard of four days out of seven on treatment to obtain a rating but will counsel the participant and family about the importance of adherence in such a situation. We will require a minimum of 5 days per week on treatment in each of the final two weeks of each treatment block, so that the end-of-treatment ratings are an accurate reflection of treatment. For the final study visit and pTMS assessment, subjects must have 100% compliance for the two days prior to testing or testing will be rescheduled within a reasonable 3-4 day window so that compliance can be assured.

Communication:

To facilitate data collection, safety monitoring, and accommodate preferences of participants, communication between study staff and the research participant/family/teachers will utilize a variety of technologies. Modes of communication may include phone calls, videoconference, electronic mail (e-mail), REDCap surveys (via e-mail link or Twilio text message), and in person meetings. Responses to safety assessments will be reviewed by a member of the study team within 48 hours. As described elsewhere, if a participant/family discloses any suicide ideation or significant health concerns at any point in this study, the study staff will contact the participant's mental health provider or primary care provider and/or Psychiatric Intake Response Center (PIRC) at CCHMC for further assessment and guidance within 24 hours.

Video recordings may aide recruitment and explanation of study assessments/details. These recordings may be distributed in a similar manner to other advertisements (websites, philanthropic/educational events, through interactions related to clinic visits, distributed via REDCap correspondence, et cetera). Examples or video content would be a short description of TMS and PANESS procedures. A general outline of the study (risks/benefits, procedures, timeline, et cetera) as would be described during informed consent would also be advantageous.

Medical/safety assessments:
During screening, children will receive a review of systems and physical examination, laboratory measures (CBC, basic chemistries with liver transaminase, thyroid function test, urinalysis), and pregnancy test (if relevant). Pregnancy tests will be administered when deemed appropriate by the study doctor or designee. Results of the pregnancy test will be provided to the parent/guardian of the participant. If an uninterpretable urine pregnancy test is obtained or if the participant cannot/refuses to provide a urine sample, then a serum pregnancy test will be performed. All values outside the reference range will be assessed by the investigator, Dr. Gilbert, for clinical relevance.

All MRI's will undergo a safety review by a radiologist in order to determine whether there are any clinically relevant incidental findings that require referral for appropriate clinical follow-up. Referrals for clinical follow-up will be provided and that an individual with appropriate clinical qualifications will inform the research subjects of the need for the clinical follow-up.

Measurement and Management of Adverse Effects:
Participants and parents/guardians will be given the opportunity to report AEs to the investigator spontaneously in response to a general prompt (e.g. "Any changes in your health since the last visit?"). Terminology for adverse events (AEs) will follow the Medical Dictionary for Regulatory Activities (MedDRA) version 20.1 (https://www.meddra.org) (**Table 6**). If AEs occur, there investigator will rate the seriousness of the event, whether the event was anticipated, and the likelihood that it is related to NAC. Serious events will be reported to the IRB within 24 hours of awareness. All other adverse events will be reviewed quarterly by the study team.

Table 6: Medical Dictionary for Regulatory Activities (MedDRA)

MedRA System organ class	**MedDRA preferred term**
Nervous system disorders	Headaches, Dyskinesias, Disturbances in initiating and maintain sleep,

Psychiatric Disorders	Anxiety symptoms, decreased or increased physical activity levels, depressive symptom
Gastrointestinal Disorders	Nausea, vomiting, constipation

Dose Modification:

Definition of Dose-Limiting Toxicity (DLT)

DLT will be defined as any of the following events that are at possibly, probably or definitely attributable to therapy. Adverse events will be graded using CTCAE v5.0.

Dose Modification

Dose reductions and/or withholding of doses must be based on the following:

- Dose reduction to half dose (35mg/kg/day) to be determined by study doctor or designee (**Table 7**)
- General dose modification guidelines for clinically significant toxicities considered related to treatment are provided in **Table 8**
- Local medical practice and regional regulatory guidance.

Table 7: NAC Dose Levels

Dose Level	Dose Schedule
0	70 mg/kg/day
-1	35 mg/kg/day

Justification for Dose Modification

Previous studies have shown oral NAC administration to humans at 35 mg/kg and 70 mg/kg produced CSF concentrations above the required biological activity in the brain. Therefore, it has been established that at 35mg/kg/day, NAC passes the blood brain barrier and would be efficacious as a neurocognitive therapy.

Table 8: General Dose Modification Guidelines

CTCAE Grade	Action and Dose Modification
Grade 1	• Continue treatment at current dose level • Monitor closely • Provide supportive care according to institutional standards
Grade 2	• Interrupt treatment if clinically indicated • Monitor closely • Provide supportive care according to institutional standards • When toxicity resolves to grade 1 or baseline, restart treatment at current dose level
Grade 3	• Interrupt treatment • Monitor closely • Provide supportive care according to institutional standards • When toxicity resolves to grade 1 or baseline, restart treatment **reduced by half**
Grade 4	• Interrupt treatment • Monitor closely • Provide supportive care according to institutional standards

	• Restart with treatment **reduced by half** once toxicity resolves to grade 1 or baseline • If the grade 4 toxicity recurs, **permanently discontinue treatment**

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit during the program. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. All adverse events must be recorded in an agreed-upon format, such as on the Adverse Event Form, which will be provided with the following information:

1. Severity grade (mild, moderate, severe)
2. Relationship to the drug(s) of interest (suspected/not suspected)
3. Duration (start and end dates or if continuing at final exam)
4. Whether it constitutes a serious adverse event (SAE)

ELECTRONIC MAINTENANCE OF THE RESEARCH REGULATORY BINDER

To securely and efficiently maintain the data collected for this study, an electronic (eREG) binder will be utilized. All data kept in computerized files are in computers or a server with restricted, password-protected access. The CCHMC network server is regularly backed up. Essential regulatory documents provided in paper format will be scanned into electronic format and filed appropriately in the eREG binder.

A study team member will verify that the resulting copy contains all the original attributes and information found in the original document and certify, after verification, the electronic version is an accurate and complete reflection of the original. Verified electronic images will be considered "substitutes" or "certified copies" of the original documents. Original documents will be destroyed in a manner that ensures the confidentiality of the records and renders the information no longer recognizable as CCHMC records.

The Principal Investigator or designee will provide approval for document conversion from paper to electronic. A Conversion Process Documentation Template will be used to document this process.