A feasibility study of N-acetylcysteine for self-injurious behavior in children with autism spectrum disorder

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A feasibility study of N-acetylcysteine for self-injurious behavior in children with autism spectrum disorder

Emory University School of Medicine
Marcus Autism Center
1920 Briarcliff Road NE
Atlanta, GA 30329

Lawrence Scahill, MSN, PhD, Professor of Pediatrics,
Nathan A. Call, PhD, Associate Professor of Pediatrics,
Mindy Scheithauer, PhD, Assistant Professor of Pediatrics,
Rabindra Tirouvanziam, PhD, Assistant Professor Pediatrics

Protocol History

Version 1.0 - Drafted Spring 2016

- Submitted to Emory IRB in Spring, 2016

Version 2.0 – Drafted 10/24/16

- Revised format, L. Scahill listed as PI
- Added Appendices A (measures) and B (human subjects section)

Version 2.1 – Drafted 12/02/16

- Fixed typographical errors.

Version 2.2 - Drafted 12/05/16

- Version sent to the FDA with initial IND application. Mentions that Mindy Scheithauer and the independent evaluator (to be named) will remain blinded in the open-label phase
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Version 2.3 – Drafted 12/09/16

- Incorporated recommendation s from FDA review in exclusion criteria (children with a history of asthma or a hypersensitivity reaction to NAC)
- Included FDA recommendation for grading adverse events
- Revised description on the clinical management of Grade 3 and 4 adverse events
- Removed Jorge Juncos, MD as a co-investigator in the protocol.

Version 2.4 - Drafted 12/13/16

- Added a comment regarding safety monitoring
- Discussed exploratory analysis of antioxidant and glutamate indices in platelet-free plasma in the Abstract

Version 2.5 - Drafted 12/13/16

- Dr. Mindy Scheithauer is listed as the independent evaluator

Version 2.6 - Drafted 12/29/16

- Revisions to Aim #3 and analytic plan for preliminary efficacy: includes pre- and post-treatment evaluation of change on the ABC-Irritability subscale, adds mention of pre- and post-treatment glutamate levels
- Minor revisions to Schedule of Measures: added CGI-Severity at Week 9, replaced Vineland II with Vineland III, noted that direct observation procedure for counting SIB is separate from the functional analysis, clarified the use of Parent Target Problem in outcome evaluation and placed description in body of protocol rather than in Appendix A

Version 2.7 - Drafted 01/04/17

- Revision to data safety and monitoring plan (procedure for breaking the blind) in Appendix B
- Revised the time line for notification of reportable Serious Adverse Events to IRB from 48 hours to 5 business days
- Noted that there are no costs to participants who enroll in the study and that the study drug and placebo will be supplied by BioAdvantex

Version 2.8 - Drafted 01/05/17

- Per suggestion of M. Huber, RN at the Emory Office of Research Compliance, added five sentences on reporting of adverse events to the FDA and the Emory IRB. Revised the reporting times for SAEs to Emory IRB from 5 days to 10 days (current IRB policy) and to FDA with 15 days (7 days if fatal or life threatening events)

- Drs. Scahill and Sidhu will review AEs quarterly and report to the Emory IRB as per policy.

Version 2.9 - Drafted 02/03/17

- Clarified in Table 2 that screening occurs over two visits

Version 3.0 - Drafted 03/03/17

- Clarified that the study database system runs on software provided by Prometheus Research. The data system is a HIPAA-compliant, doubly-encrypted, password-protected database housed and operated at the Marcus under a contract with Prometheus Research. All data entered are stored on secure servers maintained by Prometheus through a third party called Rackspace. Rackspace maintains geo-redundant data storage to protect against data loss
- Removed payment for assessment visits from protocol and the consent form
- Confirmed that Dr. Scahill is listed as PI in consent form.

Version 4.0 - Drafted 08/31/2017

- Add visit windows for all study visits
- Removed ADOS and replaced with DSM-V Checklist and SCQ
- Updated schedule of measures
- Clarified that the Short Form version of the Caregiver Strain Questionnaire will be used

Version 5.0 – Drafted 7/9/2018

- Added a blood draw protocol & criteria for if vitals and/or blood draw are not collected due to child noncompliance
- Removed ADOS from a location previously missed in last version
- Modified the consent for a few areas of clarification
- Revised Visit Windows.

Version 5.1 – Drafted 02/18/2019

- Updated the terminology of the CGSQ-SF to indicate the CGSQ will be administered (i.e., 7 item to 21 item questionnaire).
- Changed the treating clinician from Ms. Rapkin to Ms. Hesney.
- Added a skin check procedure to the functional analysis and direct observation assessments.

Abstract
Self-injurious behavior (SIB) in children with autism spectrum disorder (ASD) can cause physical harm to the child, interfere with acquisition and performance of daily living skills, hinder educational progress, increase caregiver stress, and often requires placement in restrictive settings. Research in the field of applied behavior analysis indicates that SIB in children with ASD may be maintained by external reinforcement such as achieving escape from routine demands, obtaining access to a preferred object (toy or food item) or attention from a caregiver. In other cases, SIB appears repetitive, stereotypic and without clear purpose. Behavioral interventions for SIB in children with ASD are labor-intensive, but consistently successful when the purpose or *function* of the behavior (escape, preferred object or attention) is identified. By contrast, behavioral interventions for children with repetitive SIB are often less successful. Medications from various classes have targeted SIB in ASD with mixed results – perhaps due to inclusion of children with different types of SIB. We propose a pilot feasibility study of N-acetylcysteine (NAC) in 14 children with ASD and repetitive, stereotypic SIB. NAC has antioxidant properties and available evidence also suggests that it reduces glutamate signaling in the brain. In this Phase II study, children (age 5 to 12 years) will be randomly assigned to gradually increasing doses of NAC (maximum 2.7 grams/day) or placebo for 9 weeks under double-blind conditions. Feasibility outcomes will focus on recruitment, attrition, treatment compliance, successful data collection and the accuracy of our screening procedures. Preliminary efficacy will be evaluated with laboratory counts of SIB, parent ratings, and overall improvement assessed by a clinician blind to treatment assignment. Safety will be systematically evaluated at each visit and with pre- and post-treatment analysis of routine laboratory tests. Finally, we will explore the effect of NAC on cysteine/cystine and glutathione/ glutathione disulfide (GSH/GSSG) ratios, and glutamate level and glutamate/glutamine ratio in platelet-free plasma.

SPECIFIC AIMS
Autism spectrum disorder (ASD) is a heterogeneous and often disabling condition affecting as many as 14 per 1,000 children. Self-injurious behavior (SIB), aggression or both are common in youth with ASD affecting at least a third in this age group (Mazurek et al., 2013). In clinical practice, treatment of children with ASD accompanied by SIB commonly combines behavioral intervention and drug therapy. To date, however, most research has focused on behavioral **or** pharmacological intervention. With the notable exception of our previous study that compared risperidone alone to risperidone plus parent training, studies of combined treatments in children with ASD are few in number (Aman et al., 2009; Scahill et al., 2012). In this application, we describe a first step toward building a research program that will combine behavioral assessments and interventions with pharmacology to test new treatments in children with ASD and SIB. We propose a pilot study of N-acetylcysteine (NAC) to demonstrate the feasibility of our behavioral assessment methodology and to evaluate the preliminary efficacy of NAC for reducing self-injurious behavior (SIB) in children with ASD.

NAC is an *over the counter* dietary supplement. Given orally or IV, NAC has been used to treat acetaminophen overdose for many years and has been used as an aerosol to reduce viscosity of mucus secretions in cystic fibrosis. Oral NAC has also been tested in cystic fibrosis in single (Tirouvanziam et al., 2006) and multi-center trials (Conrad et al., 2015). Oral NAC functions as a prodrug for the amino acid cysteine. Following an oral dose, NAC is readily absorbed through the gut epithelium, the N-acetyl group is cleaved by cytosolic esterases and cysteine is transported via the portal route to the liver. In the liver, cysteine is used to synthesize glutathione (GSH), which is then available systemically. In acetaminophen overdose, NAC replenishes depleted GSH in the liver and protects against oxidant injury. Oral administration of NAC also presumably enhances GSH availability in the brain, which enhances antioxidant activity in brain (Berk et al., 2013). Oral NAC, via increased GSH and cysteine may also reduce synaptic release of the excitatory neurotransmitter, glutamate (Villagonzalo et al. 2010). Oxidative stress and abnormal glutamate signaling have been proposed in the pathophysiology of ASD (Tirouvanziam et al., 2012; Berk et al., 2013; Frye et al., 2014). Thus, the mechanism of action of NAC may be relevant to ASD. Increasing interest in NAC is also reflected by the growing number of studies in schizophrenia, bipolar disorder, depression, trichotillomania and ASD (reviewed in Berk et al., 2013). These studies indicate that NAC is generally well tolerated in adults and children. Common adverse effects include nausea, vomiting and diarrhea. Acute allergic reactions have been reported – but are rare and associated with IV treatment in acetaminophen overdose. A pilot study of 29 children (age 3 to 10 years) with ASD provides promising preliminary results for reducing parent-rated

disruptive behavioral problems (tantrums, aggression and self-injury) in children with ASD (Hardan et al., 2012). The impact of NAC on SIB was not reported.

Under the direction of Dr. Nathan Call, the Severe Behavior Unit at the Marcus Autism Center has extensive experience in the application of specialized behavioral assessment and treatment of SIB in children with ASD. This work, based on the principles of applied behavior analysis, considers the purpose or *function* of SIB when selecting treatment components for a child. Through the careful analysis of function (see below), we classify SIB that is reinforced by social reward such as allowing escape from a routine demand, or obtaining a preferred object (a food or a toy) or gaining caregiver attention. Alternatively, we classify SIB as a repetitive, *automatic* behavior that is somehow self-reinforcing. The techniques of applied behavior analysis are predictably more successful for reducing SIB maintained by *social* reward than *automatically* maintained SIB. This observation suggests that neural pathways that underlie habitual *automatically* maintained SIB may be different from those underlying socially maintained SIB. This study is focused on automatically maintained SIB.

Aim #1. To demonstrate the feasibility of conducting a 9-week, randomized, double-blind trial of NAC compared to placebo in 14 children (age ≥ 5-≤ 12 years 11 months) with ASD and moderate level of SIB
Feasibility includes demonstrating successful recruitment of eligible subjects for a randomized, placebo-controlled study of NAC, that the treatment is acceptable to parents, and successful collection of study outcome measures. **Benchmarks include:**
a) demonstrate capacity to screen 2-3 subjects/month (in order to average 1.75 randomize subjects/month);
b) no more than 15% attrition rate (e.g., no more than 2 of 14 randomized subjects);
c) at least 70% compliance with study medication (determined by tablet counts and drug dairies).
d) at least 80% collection of essential outcome measures (actual ratings ÷ expected X 100).
e) at least 80% of parents will *agree* or *strongly agree* when asked in an anonymous survey that they would recommend the study and the study treatment to other parents of children with ASD and SIB.

Aim #2. To evaluate the positive predictive value of our screening method to classify children with automatically maintained self-injurious behavior.
The definitive method of classifying SIB as *socially* maintained or *automatically* maintained is a specialized five- to six-hour assessment called a *functional analysis*. We intend to enrich the study sample with children classified with automatically maintained SIB based on the functional analysis. To minimize subject burden and to manage study resources, we want to avoid conducting the detailed functional analysis on subjects who do not get classified with automatically maintained SIB. We have developed a screening interview to aide in identification of subjects with automatically maintained SIB before undertaking the detailed functional analysis. Demonstrating a high positive predictive value is a necessary prerequisite for launching a larger study. Using the formula: Positive Predictive Value (PPV) = screen positive and true cases ÷ all positive screens, we set a benchmark of 75%. Information learned in the study will likely enhance our PPV for future studies.
NOTE: Children who are classified with socially maintained SIB will not be randomized. Dr. Call will meet with the family to plan the next phase of treatment.

Aim #3. To evaluate the preliminary efficacy of NAC for reducing SIB in 14 children with ASD in a 9-week, double-blind, placebo-controlled, randomized trial.
This sample size is not sufficient to test the superiority of NAC over placebo. We use a randomized design to meet Aim #1 and to enable blinded assessment of outcome by an independent evaluator.
Preliminary efficacy benchmarks within the NAC group
a) Significant decline from baseline to Week 9 on the parent-rated Aberrant Behavior Checklist Irritability subscale (commonly used parent-rated survey of tantrums, aggression, and self-injury).
b) at least 50% decline (on average) from baseline to Week 9 in the number of SIB events in children treated with NAC when counted by a trained technician who is blind to treatment assignment.

c) at least 50% of the subjects on will be rated *much improved* or *very much improved* on the Improvement scale of the Clinical Global Impression by a clinician blind to treatment assignment.

Aim #4. To evaluate the safety and tolerability of NAC in children with ASD. The treating clinician (Ms. Sarah Hesney, CPNP) will systematically monitor adverse events at every visit. The occurrence of an AE will be counted once at the highest level of severity (e.g., a report of mild nausea followed by a report of moderate nausea in the same child would be counted as an occurrence of moderate nausea).

Aim #5. To evaluate biomarkers and possible mechanisms of action of NAC in children with ASD.
To support a future NAC trial in a larger sample, we will investigate potentially relevant redox and amino acid levels before and after treatment. Given the sample size, we do not expect differences between groups. Within the NAC group, we expect increased cysteine/cystine and glutathione/glutathione disulfide (GSH/GSSG) ratios, and decreased glutamate and glutamate/glutamine ratio and increased GABA levels in platelet-free plasma.

BACKGROUND & SIGNIFICANCE
The number of children **identified** with ASD has increased over the past two decades. The most recent survey by the Centers for Disease Control reported a prevalence of 14.7 per 1,000 children and estimated that 31% of children with an ASD are intellectually disabled (Centers for Disease Control, 2014). This compares to the estimate of 3.4 per 1,000 in 2003 (Yeargin-Allsopp, 2003). This increase reflects a broadening of diagnostic criteria and increased awareness of ASD. Given the expanded definition, it is clear that ASD is a heterogeneous disorder that ranges from mild to severe. In addition to the core features, children with ASD may have serious behavioral problems including tantrums, aggression and self-injury. In a sample of 1,584 children with ASD (age 2-17 years) not selected for behavioral problems, 53.7% had a current or past history of aggression and 36% with SIB based on parental response (Mazurek et al., 2013).
We evaluated subtypes of aggression and SIB in 200 subjects with ASD and serious behavioral problems (168 boys, 32 girls; mean age = 8 years). These children participated in one of two Research Units on Pediatric Psychopharmacology (RUPP) Autism Network risperidone trials (Carroll et al., 2014; RUPP Autism Network 2002; Aman et al., 2009). Five subtypes emerged: aggression occurring in the context of tantrums (32.5%), aggression without tantrums (16%), SIB only (16.5%), aggression and SIB (8.5%), and non- aggression (26.5%). Children with IQ < 70 were more likely to show SIB – alone or in combination with aggression. In some cases, parents described the SIB as repetitive in nature and without clear purpose (Carroll et al., 2014).

In summary, SIB may be mild (occasional without associated tissue damage), moderate (sometimes to often with some tissue damage), severe (often with tissue damage and potential disfigurement). Moderate or greater SIB hinders educational and habilitative interventions, interferes with social interaction, adversely affects family functioning, may contribute to marital discord, and often leads to placement in restrictive school settings or hospitalization. Inability to make use of educational and habilitative interventions limits the child's overall development and reduces the likelihood of independent living.

Treating SIB
In clinic, there are two approaches to treating SIB in children with ASD: behavioral intervention and drug therapy. Two medications (risperidone and aripiprazole) are FDA-approved for the treatment of children with ASD. FDA-approval for these medications was based on their demonstrated superiority to placebo on the Irritability subscale of the Aberrant Behavior Checklist. This 15-item parent rating includes tantrums, aggression and self-injury (RUPP Network, 2002; Owen et al., 2009). To date, however, medication studies specifically targeting SIB in children with ASD have been disappointing. These disappointing results may be due to drug selection, sample selection, selection of outcome measure, or all of the above.

Behavioral interventions begin with a detailed assessment of the purpose or *function* of SIB. In this model, the child may engage in SIB because the environment reinforces the behavior. Parents faced with managing SIB may provide the child's preferred object (specific toy or food item), allow the child to escape a routine demand (getting dressed) or

provide attention and comfort to reduce the behavior. Over time, the child *learns* that engaging in SIB consistently produces these social rewards, which makes the behavior more likely to occur in the future. In some children, however, the behavior appears repetitive and *automatic.* For these children, it is difficult to identify what is reinforcing the SIB.

Experience with Behavioral Treatment of Aggression and SIB in ASD

The **Severe Behavior Unit at Marcus**, directed by Dr. Nathan Call, offers intensive day treatment (6 hrs/day for 12 weeks), outpatient clinical services (weekly visits for one or two hrs) and home/community-based interventions (10 weekly, two-hour sessions). This program is one of the few programs in the country with the capacity to provide this range of behavioral interventions for children with ASD and serious behavioral problems (Alvarez, Call, & Lomas Mevers, 2001; Call, Parks, & Reavis, 2013; Scheithauer, Lomas Mevers, Call, & Shrewsbury, submitted). In 2014 our program treated a total of 293 children with significant aggression, SIB, or both. The day-treatment program treated 41 patients with ASD or other developmental disabilities (mean age=11 years; range 4-21) accompanied by extreme SIB, aggression and property destruction. The home-based program served 121 children with ASD and moderately severe behavioral problems. The weekly outpatient program treated 131 children (mean age = 8.6 years; range 3-20) with disruptive behavior who did not need the more intensive day treatment or home-based treatment. Referral pressure to our clinic remains high with a current waiting list of 433 children. Of these, we estimate that 40 subjects between age 5 and 132 with a primary complaint of SIB are on the waiting list.

INNOVATION

The Severe Behavior Unit at the Marcus specializes in the behavioral treatment of aggression, self-injury and disruptive behavior. Drs. Call and Scheithauer conduct detailed behavioral assessments on a daily basis to pinpoint the *function* of SIB and create individualized treatment plans.

This project will be the first study at Marcus to apply these sophisticated behavioral assessment methods in a pharmacological trial. In addition to expertise in behavioral assessment and intervention, the project includes expertise in the design and conduct of clinical trials (Dr. Scahill). Dr. Scahill has an extensive track record of NIMH-funded clinical trial experience. Demonstrating the feasibility of these methods borrowed from behavior therapy for early drug development is a necessary prerequisite for obtaining external funding. In addition to expertise in behavioral intervention (Drs. Call and Scheithauer) and clinical trials (Dr. Scahill), our team includes Dr. Rabindra Tirouvanziam, Assistant Professor of Pediatrics. Dr. Tirouvanziam is a pioneer in the use of NAC in cystic fibrosis (Tirouvanziam et al., 2006; Conrad et al., 2015) and ASD (Hardan et al., 2012). He also has experience measuring redox metabolites and amino acid neuromediators in children with ASD (Tirouvanziam et al., 2012). We will also benefit from ongoing consultation from Dr. Jorge Juncos, Associate Professor of Neurology at Emory, who has extensive clinical experience using NAC in the treatment of SIB in adults with ASD. We will have medical oversight from Dr. Nareet Sidhu, the Medical Director at Marcus.

Marler and colleagues (2014) provided encouraging results in a case report of a four-year-old boy with ASD and SIB at a dose of 1800 mg/day. To our knowledge, however, this will be the first randomized study of NAC in children with ASD focused on repetitive SIB. We have selected NAC based on its antioxidant and glutamate-modulating properties. Preclinical and neuroimaging data have shown that glutamatergic cortical-striatal pathways play a role in a range of repetitive behavior including addiction, gambling and compulsive grooming behaviors (Kalivas and Volkow, 2005; Graybeil, 2008). In addition to its well established antioxidant properties, NAC appears to decrease in glutamatergic signaling in the brain, which may be relevant to repetitive behavior (Berk et al., 2013; Odlaug & Grant, 2007). Given the evidence that glutamatergic signaling plays a role in repetitive behavior, we will test the feasibility of enrolling children with *automatically* maintained SIB.

APPROACH

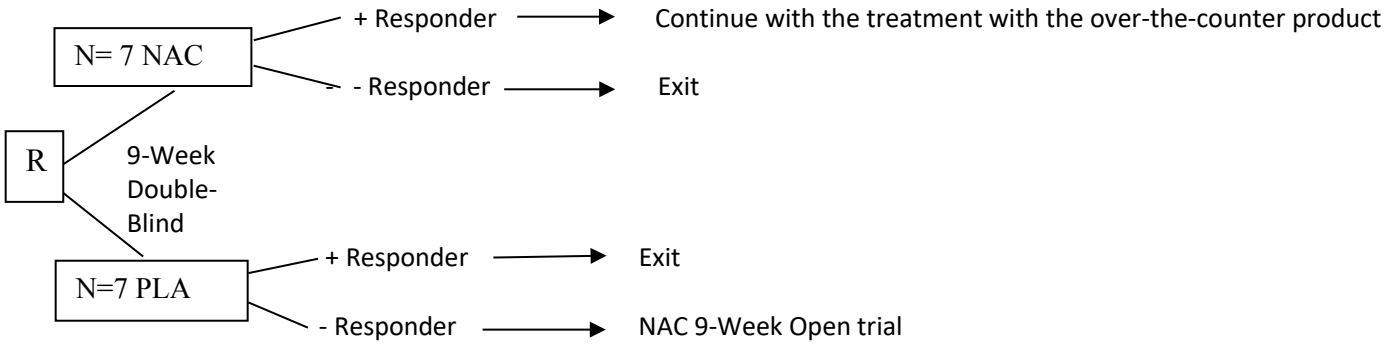
Design

As shown in Figure 1, subjects will be randomized 1:1 to 9 weeks of NAC or placebo. Randomization to NAC or placebo will be 1:1 using permuted blocks without stratification. The permuted blocks will be constructed and managed by the

Investigational Pharmacist (Dr. Jim Rhodes). The allocation pattern will be concealed to investigators. Dr. Rhodes will receive a form via email from the coordinator indicating that the subject meets entry criteria and he will assign the subject to NAC or placebo according to the permuted block.

Each subject will be followed by two blinded clinicians. The treating clinician (Ms. Sarah Hesney, CPNP) will monitor adverse events and manage the dose of the study medication. The independent evaluator will assess therapeutic response and will not discuss adverse events or medication dose with parent and child.

Figure 1. Study design



Dosing Active NAC and matching placebo will have identical appearance, odor, and taste. The effervescent tablet formulation of NAC and matching placebo will be supplied by BioAdvantex Pharma Inc. from Ontario, Canada (see attached letter from the company).

Participants will start with a single dose of 900mg once daily for one week. At Day 7, parents (primary caregiver) and subjects will return to Marcus for adverse event review. In the absence of dose limiting adverse events attributable to the study drug, the dose will be gradually increased (Day 8) to 900 mg twice a day. If

Table 1. NAC dose schedule for 9-week study in mg					
Study Day 1-7	900 QD	**Study Day 8-28**	900 BID	**Study Day 29 -63**	900 TID

this dose is well-tolerated, the dose will be increased to 900 three times per day. This dose schedule is adapted from the NAC trial in children with ASD (Hardan et al. 2012). This dose schedule serves as a guide and is not fixed. The treating clinician (Ms. Hesney, CPNP) can delay a scheduled increase or reduce the dose to manage an adverse effect at any time.

The treatment mask will be broken at Week 9 by Dr. Call (Director of the Severe Behavior Clinic) who will not be involved in management of participants during the trial. Ms. Hesney will follow placebo non-responders in the Open-label trial. She will remain blinded to the results of those subjects randomized to NAC. The independent evaluator, Dr. Scheithauer, will remain blinded.

Aim #1 [Feasibility]

Pilot studies are not about efficacy (Leon et al., 2011). Before launching a larger **definitive** randomized clinical trial, we need to show that the study procedures and medication are acceptable to parents, that we can successfully recruit and retain children with moderate to severe repetitive SIB in a randomized trial, and that we can collect outcome data. Fourteen children with ASD will be enrolled and randomly assigned to NAC or placebo for 9 weeks. Enrollment will occur from Month 2 through Month 9 of the project (average at least 1.75 subjects per month). Children will be recruited through the Marcus Autism Center (from the current waitlist and referrals). Potentially eligible children will be screened by phone to confirm the presence of SIB that is moderate – but not in need of immediate service. Children with presumably moderate SIB will be invited to come to Marcus for a formal screening visit to confirm eligibility (see Table 2 for Schedule of Measures).

Table 2.0 – Schedule of Measures

					Study Week				
Measures	**Staff**	**Screen**	**FA Screen**	**Baseline**	**1**	**2**	**4**	**6**	**9**
ABC	RC	X		X			X		X
Adverse Event	MD	X		X	X	X	X	X	X
CGI-I	IE			X			X		X
CGI-S	IE	X		X			X		X
CGSQ	RC			X			X		X
Cognitive Test (Mullen or Stanford-Binet 5)	IE	X							
CYBOCS-ASD	IE			X			X		X
Demographic Form	RC	X							
Detailed Functional Analysis	IE/RC		X						X
Direct Observation Session	IE/RC			X					X
Drug Compliance	RC				X	X	X	X	X
DSM-V checklist	IE	X							
Functional Assessment Interview	IE	X							
Medical History	IE	X							
Parent Satisfaction Questionnaire	RC								X
Parent Target Problem	IE	X		X			X		X
Physical Exam	MD	X							
Research lab test**	RC	X							X
Responder Status	IE								X
Routine blood tests*	RC	X							X
Safety Review	MD	X		X	X	X	X	X	X
SCQ	RC	X							
Treatment Review and Planning	Clinic								X
Vineland III	RC	X							
Vitals, Height and Weight	RC	X		X			X		X

* CBC, electrolytes, BUN, creatinine, liver function tests
** see Aim 5 (drawn at same time points of safety labs)

Subject Characterization Subjects will be characterized in accordance with current practice at Marcus to confirm an ASD diagnosis and essential clinical characteristics (e.g., IQ, adaptive functioning, behavioral problem profile). For details, see Appendix A.

Inclusion criteria

1. Boys and girls ≥ 5 and ≤12 years 11 months
2. Confirmed diagnosis of ASD (clinical interview, Social Communication Questionnaire, DSM-V checklist)
3. Medication- free or stable medication (at least four weeks) with no planned changes for the 9-week trial.
4. Anticonvulsants for seizures will be allowed if the dosage has been stable for 4 weeks and the subject has been seizure free for at least 6 months.
5. Presence of SIB classified as automatically maintained, based on functional analysis (described below).

Exclusion Criteria

1. Presence of a known serious medical condition in the child (based on medical history) that would interfere with child ability to participate in the study.
2. Presence of a current serious behavioral problem or psychiatric condition in the child that would require another treatment (e.g., psychotic disorder, major depression) (based on all available information collected at screening). [RATIONALE: children with a serious, untreated psychiatric condition should proceed with treatment for that psychiatric condition.]
3. Prior failed treatment of NAC of at least 1200 mg per day for at least 4 weeks.
4. Presence of unacceptable adverse effects, including but not limited to hypersensitivity reactions, with prior treatment with NAC at any dose.
5. Children with a history of asthma
5. On a psychotropic medication, but regimen is not stable. [RATIONALE: For children on unstable medication regimen it may be difficult to interpret efficacy and/or tolerability of the study treatment. Once stabilized, eligibility could be reconsidered.]

Visit Windows

The visit windows for each assessment visit are:

- FA Screen is -0/+14 days from initial Screen
- Baseline is -0/+14 days from FA screen
- Week 1 is 7 -3/+3 days from Baseline
- Week 2 is 14 -5/+5 days from Baseline
- Week 4 is 28 -7/+7 days from Baseline
- Week 6 is 42 -7/+7 days from Baseline
- Week 9 is 63 is -9/+9 days from Baseline

Aim #2 [Positive Predictive Value]

The screening visit will also include a semi-structured interview to make a **preliminary** determination whether the repetitive behavior is *socially maintained* or *automatically maintained*. Subjects that are classified with a preliminary classification of *automatically maintained* SIB will be invited to participate in the trial and proceed to the baseline visit. We expect to evaluate 18-20 children to randomize 14 eligible and willing subjects.

Functional Analysis Under the direction of Dr. Scheithauer, the FA screen visit will include the detailed functional analysis to confirm whether the SIB is primarily *socially* or primarily *automatically maintained*. In the functional analysis, we manipulate antecedents (situations or events that trigger the child's SIB) and consequences (response of the environment) to identify the purpose of the behavior. For example, to confirm that SIB is maintained by access to a preferred object (an iPad), the child would be allowed to play with an iPad for a period of time – then the therapist removes the iPad to observe the child's reaction. If the child engages in SIB, the iPad is given back to the child to see if the SIB stops. This sequence is an analog to the home situation in which a caregiver gives the child a preferred item in order to reduce SIB. To test whether SIB is maintained by escape from routine demand, the therapist would attempt to engage the child in a non-preferred task (age and developmentally appropriate section of an IQ test). If the child engages in SIB, the therapist would permit a break in the demand to observe the child's response. The functional analysis also includes a session in which the child has access to parent-identified preferred items (enriched environment) and a session in which the child is alone (no one is available to provide a social reward). The frequency of SIB is tallied, graphically displayed as counts per minute and compared across test conditions (demand, attention, tangible item, alone) to the control condition (enriched environment). Higher counts of SIB in a specific test condition compared to the control condition will be used to verify what is reinforcing the SIB (e.g., getting attention, access to a tangible item, or escape from a demand). For example, if the child engages in SIB when asked to do work, but not during the free play when no demands are presented, the SIB is reinforced by escape from demand. To identify whether SIB is *automatically maintained*, we will assess whether the child alone condition (no social antecedents or consequences present) is elevated over the control condition. This entire sequence may be repeated to confirm the function of the behavior. We expect the functional analysis to take 5-6 hours. A skin check is performed following sessions with moderate levels of self-injurious behavior, which will document marks likely caused by the behavior observed in a session. The child's visible body parts (i.e., arms, face) and any targeted body parts (e.g., back, scalp) will be assessed. We acknowledge that a few children with a preliminary classification of *automatically maintained* SIB may be reclassified as *socially maintained* SIB following the detailed functional analysis. Because the functional analysis is the gold standard for confirming the purpose of the SIB, **these subjects will be excluded from the study and advised on treatment options**. We do not expect that this reclassification will occur often. For example, if the screen achieves our benchmark of 75% positive predictive value (PPV), 4 of 14 subjects would be reclassified as *socially maintained* SIB and excluded. An important aim of this pilot study is to identify ways to maximize our PPV for future studies.

Aim # 3 [Preliminary Efficacy]

Study outcome measures are listed in Table 2. All outcomes including direct observation of behavior in the laboratory setting, parent ratings of behavior and assessment of overall improvement will be collected blind to treatment assignment. Here we describe the outcome measures that are directly related to preliminary efficacy benchmarks (see Appendix A for detailed description of other outcome measures).

Aberrant Behavior Checklist (ABC) is a 58-item, informant-based scale with five subscales: *I. Irritability* (includes agitation, aggression and self-injurious behaviors, 15 items); *II. Lethargy* (includes social withdrawal, 16 items); *III. Stereotypic Behaviors* (7 items); IV. *Hyperactivity*, 16 items (includes overactivity and impulsiveness); and *V. Inappropriate Speech* (4 items). The ABC has been used as an outcome measure in many studies of children with ASD (RUPP Autism Network, 2002; RUPP Autism Network, 2005; Aman et al., 2009; Hardan et al., 2012; Bearss et al., 2015). In this proposed study, the parent-rated Irritability subscale will be of particular interest because it includes items on SIB. We will also be interested in the Stereotypy subscale.

Direct observation of the frequency and severity of SIB will also be collected at baseline and endpoint at the conclusion (but separate from) of the functional analysis. Operational definitions will be created for each child based on the parental description of the SIB. For example, if the child engages in hand-to-head hitting, the operational definition may be anytime the child's hand comes in contact with the head from a distance of 6-inches or greater. Similarly, for a child who engages in hand-biting the definition may be when the child's teeth make contact with the hand. Frequency data will be collected for any discrete behavior (such as head banging) and duration data will be collected for behaviors that

may occur for longer periods of time (such as hand biting). Real-time counts of SIB will be tallied by a trained rater (blind to treatment condition) during a set of structured, 10-minute conditions that will include routine demand, restricted access to tangible items, restricted access to attention, free play with free access to parent-identified preferred items, and when the child is left alone in an austere environment. The rater will also complete a 4-point severity rating scale of SIB (0=none, 1=mild, 2=moderate, 3=severe) for each condition. A skin check is performed following sessions with moderate levels of self-injurious behavior, which will document marks likely caused by the behavior observed in a session. The child's visible body parts (i.e., arms, face) and any targeted body parts (e.g., back, scalp) will be assessed. Data collectors will denote the severity rating based off of the skin checks. Per minute counts of SIB across the entire length of the observational period as well as per minute counts in different conditions (routine demands, restricted access to tangible items, restricted access to attention, free play, and alone in austere environment). An average of the 10-minute severity ratings will also be calculated at baseline and endpoint. Change in counts and the average severity score within the NAC group (paired t test) and across treatment groups (ANCOVA) will be used to evaluate preliminary efficacy. Counts and severity during the alone condition (automatically maintained SIB) will be analyzed separately.

Parent Target Problems At baseline, parents will be asked to nominate the two most important problems for the child. Through brief discussion, the frequency (for episodic behaviors) or constancy (for problems such as impulsiveness reflecting more enduring patterns), intensity and impact of the behavior on the family are established. Responses from this systematic inquiry are documented in a brief narrative. The narrative will be reviewed at Weeks 2, 6 and 9 and a new narrative documented. This review will assist with the scoring of the CGI-I in real time (Scahill et al., 2015).

Improvement item on the Clinical Global Impression scale (CGI-I) The CGI-I is a 7-point scale designed to measure overall improvement from baseline (Scahill et al., 2015). Scores range from 1 (Very Much Improved) to 4 (Unchanged) to 7 (Very Much Worse). The CGI-I will be used to assess overall response to treatment. By convention, scores of Much Improved or Very Much Improved are used to define *positive response*; all other scores are classified as a *negative response*. Using available information (ABC scales, Parent Target Problems, Parent Daily Rating), the CGI-I will be rated by a blinded independent evaluator (Dr. Scheithauer) trained by Dr. Scahill.

Aim #4 [Safety and Tolerability]

A blood sample for routine lab tests (CBC, BUN, creatinine, liver function panel) will be collected at screening and at endpoint. These lab results will be reviewed by Ms. Rabin (Pediatric Nurse Practitioner) with consultation from Drs. Scahill and Sidhu as needed. Clinically important findings will be shared with the family.

Adverse Events At each visit the treating clinician will ask specific queries about major body systems, activity level, sleep, appetite, and general health. The assessment will be guided by the Adverse Events Review. This measure will be conducted at baseline so that current health complaints are known and documented. New events, whether presumed related to treatment or not, are classified as an adverse event and rated as Mild, Moderate or Severe (See Appendix B for definitions).

Aim #5 [Biomarkers and Mechanism of Action]

In addition to the blood drawn for safety monitoring, we collect an additional tube (2 cc, lavender top) of whole blood to examine redox couples and amino acid neuromediator levels before and after NAC treatment. Samples will be centrifuged twice to obtain platelet-free plasma. These samples will be analyzed in Dr. Tirouvanziam's lab using ultra-sensitive, quantitative mass spectrometry methods (Tirouvanziam et al., 2006; Tirouvanziam et al., 2012; Hardan et al., 2012; Conrad et al., 2015). NAC has been shown to affect cysteine/cystine and glutathione/glutathione disulfide (GSH/GSSG) redox couples in blood, as well as levels of glutamate, glutamine and possibly, GABA. Measurement of these indices may inform the possible mechanisms of action of oral NAC (e.g., rebalancing key redox metabolites and/or excitatory/inhibitory amino acids) that may be relevant in the treatment of SIB in children with ASD.

Endpoint and Disposition As shown in Table 2, we will conduct a detailed set of outcome (direct observation, parent ratings, clinician ratings) and safety measures (Adverse Event Review, routine labs). Dr. Scahill will train the independent evaluator to rate overall response on the Improvement item on the Clinical Global Impression scale (CGI-I). The child and

parent(s) will meet with Dr. Call (Director of the Severe Behavior Clinic), who will break the blind and discuss the next steps in the child's treatment plan (see Figure 1). Children who show a positive response to NAC can continue with the treatment with the over-the-counter product. Children who were on placebo and did not show a positive response will be offered open-label NAC using the same study procedures. Children who did not show a positive response to NAC or those who showed a positive response to placebo will be advised on a case by case basis.

Data Management

Data Verification and Security: The Marcus Informatics and Analytics Core has a sophisticated data management system called DEX. This is a HIPAA-compliant, doubly-encrypted, password-protected database housed and operated at Marcus through a contract with Prometheus Research. All data entered are stored on **secure** servers maintained by Prometheus through a third party called Rackspace. Only pertinent study staff will have access to patient and study-related information. The progress of data collection will be monitored with web-based electronic data form reports, which produces a profile of all forms expected and received for each study subject. Missing-forms reports are available and are electronically accessible by the data manager and the coordinator. We will request reports on missing data, recruitment and compliance every other week. These audits are conducted by the data manager assigned to this study. Outliers and unusual values will be checked for accuracy. Data questions or problems will trigger queries back to the coordinator to assure that all forms are entered and available for analysis. Recurring problems with the data entry system will be resolved via discussion between the data manager, coordinator and PI as necessary. Prometheus contracts with Rackspace to ensure geo-redundant data storage such that in a disaster of the data center that hosts the application, no data will be lost. Nightly system backups are archived for seven days; weekly backups are archived for four weeks; monthly backups are archived for as far back as space will allow. Incremental (binary) backups of all database changes occur every 15 minutes; the write-ahead log monitors incremental changes in the interim and increases backup frequency under heavy usage.

Steps to limit attrition and missing data: In our recently completed six-month randomized trial of Parent Training in young children with ASD (MH081148; Scahill, PI) attrition was less than 10% (17 of 180) (Bearss et al., 2015). To minimize attrition and missing data in this study, we will apply similar procedures. First, during the consent process Dr. Scheithauer will engage parents in a direct discussion about random assignment, the 9-week duration of the trial, and the opportunity to receive NAC for subjects initially assigned to Waitlist control. This discussion allows expression of parental misgivings about the study and clarity about the study demands. Second, we distinguish between families who drop out completely and those who agree to return for assessments and remain on study through Week 9 – even if the family drops out of treatment. Thus, in keeping with the intent-to-treat principle, we will encourage families who encounter barriers to continue study participation and remain on study for assessment. For families who indicate intention to drop out completely from the trial, we will conduct an early termination visit to ensure a post-randomization assessment of primary and key secondary outcomes (Wisniewski et al., 2006).

Plan for Missing Data: Given the small sample size, the relatively brief trial and the offer of supervised treatment with NAC for placebo non-responders, we do not expect a high rate of attrition in either group.
Nonetheless, prevention is the first line for minimizing missing data. Upon entry, alternative contacts will be identified for all subjects to minimize loss. Timely data entry combined with bi-weekly missing data reports will prompt tracking down missing outcome assessments. Despite these prevention efforts, missing data will occur. Missing data will be checked for data entry errors. All reasons for drop out and missing data will be documented. The pattern of missing data due to drop outs will be examined by comparing subjects with missing data to subjects with no missing data.

Data Analysis

The statistician, Mr. Scott Gillespie, will conduct initial analyses to inspect data for errors, frequency, pattern, time to drop out, and missing data. This will include examination of frequency tables and dot plots for univariate data and scatter plots as needed. Data anomalies and outliers will be identified and addressed in coordination with the PI and data manager. Descriptive statistics will be calculated at baseline and follow-up time points using means and standard deviations, medians and interquartile ranges, or frequencies and percentages as appropriate.

Aim #1: As noted above, the primary aim of this pilot trial is feasibility. We set forth several benchmarks:
a) demonstrate capacity to screen 2 subjects/month (in order to average 1.75 randomize subjects/month); b) no more than 15% attrition rate (e.g., no more than 2 of 14 randomized subjects); c) at least 70% compliance with study medication (determined by tablet counts and drug dairies); d) at least 80% collection of essential outcome measures (actual ratings ÷ expected X 100); e) at least 80% of parents will *agree* or *strongly agree* when asked in an anonymous survey that they would recommend the study and the study treatment to other parents of children with ASD and SIB.

Aim #2: 75% positive predictive value (PPV = screen positive and true cases ÷ all positive screens) for our screening method to classify children with automatically maintained self-injurious behavior.

Aim #3: The use of placebo-control in this trial permits blinded assessment. These benchmarks will be examined **within** the NAC group.
a) Test for significant decline from baseline to Week 9 on the parent-rated Aberrant Behavior Checklist Irritability subscale using a paired t-test.
b) at least 50% decline (on average) from baseline to Week 9 in the number of SIB events in children treated with NAC when counted by a trained technician who is blind to treatment assignment.
In addition to percent change, we will run ANCOVAs on these outcomes within the NAC group and across treatment groups with baseline values in the model.
c) at least 50% of the subjects randomized to NAC on will be rated *much improved* or *very much improved* on the Improvement scale of the Clinical Global Impression by a clinician blind to treatment assignment.
The other outcomes listed in Table 2 above (Parent Daily Checklist, CYBOCS-ASD) will be performed as exploratory outcomes.

Aim #4: Adverse events (AE) will be systematically monitored using a 34-item Adverse Events Review form that was developed by the RUPP Autism Network to assist with the elicitation and assessment of adverse effects. It includes specific queries about major body systems, activity level, sleep, appetite, and general health. New events, whether presumed related to treatment or not, are classified as AEs and rated as Mild, Moderate, or Severe (see Appendix B). The occurrence of an AE will be counted once at the highest level of severity (e.g., a report of mild nausea followed by a report of moderate nausea in the same child would be counted as an occurrence of moderate nausea). Given the small sample size, it is unlikely that any AE will be significantly more common in NAC compared to placebo. Nonetheless, we will evaluate the frequency of AEs across groups using a Fisher's exact test (classification and reporting of AEs is described in Appendix B).

Change in direct observation measures from baseline to Week 9 will be evaluated using paired t-tests within the treatment groups and ANCOVAs across groups as previously described. Secondary measures will be assessed using longitudinal linear regression models and consider change in measure scores both within and across study groups over time.

Aim #5: Aim #5. To evaluate biomarkers and possible mechanisms of action of NAC in children with ASD.
Given the sample size, we do not expect to observe differences between groups. Using mass spectrometry, we expect increased cysteine/cystine and glutathione/glutathione disulfide (GSH/GSSG) ratios, and decreased glutamate/glutamine ratio and increased GABA levels in the NAC-treated group. These assays will use < 2 ml of blood collected at screening and endpoint when blood samples for safety are also being collected.

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Appendix A Measures
Characterization

Social Communication Questionnaire (Lifetime) (SCQ) This 40-item survey evaluates communication skills and social functioning in children who may have autism spectrum disorder. The *Lifetime Form* focuses on the child's entire developmental history, providing a Total Score that can be compared to normative data using specific cutoff points. It shows high agreement with the much longer interview called the Autism Diagnostic Interview – Revised. The SCQ can be completed by the parent or other primary caregiver in 10-15 minutes.

Intellectual Functioning Children with ASD range from severely retarded to above average IQ. We will use one of two tests: the Abbreviated Stanford-Binet V, or the Mullen (for low functioning children). The examiner will attempt to complete the Abbreviated Stanford-Binet V. If the child is unable to complete the Abbreviated Stanford-Binet V, the examiner will administer the Mullen.

Vineland Adaptive Behavior Scales (Vineland III) (Interview) assess adaptive functioning across several domains including Socialization, Communication, Daily Living Skills, and an Adaptive Behavior Composite scale, which is derived from the other three scales. The Vineland II relies on an informant (mother or primary caretaker) to provide information about the child's adaptive behavior – i.e., what the child actually *does* in the course of daily living.

Clinical Global Impression - Severity (CGI-S) This is a 7-item scale ranging from a score of 1 for "Normal" to 7 for "Extreme." In the RUPP Autism Network, we have developed a high degree of reliability with the CGI-S. Although SIB will be given particular weight in this study, independent evaluators will consider all aspects of the child's condition to assign the CGI-S score.

Adverse Events Review This 34-item form was developed by the RUPP Autism Network to assist with the elicitation and assessment of adverse effects. The treating clinician will ask specific queries about major body systems, activity level, sleep, appetite, and general health. The Adverse Events Review will be conducted at baseline so that current health complaints are known and documented. New events, whether presumed related to treatment or not, are classified as an adverse event and rated as Mild, Moderate or Severe (See Appendix B for definitions).

Outcome Measures

Direct Observation (described in the body of the protocol)

Aberrant Behavior Checklist (ABC) (described in the body of the protocol)

Clinical Global Impressions-Improvement Scale (CGI-I) (described in the body of the protocol)

Parent Target Problems (PTP) (described in the body of the protocol)

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition checklist (DSM-V checklist) lists the criteria for ASD that are outlined in the DSM. The interviewer will go through the checklist and ask the caregiver if each of the symptoms is present in the child and will mark the form accordingly.

Children's Yale-Brown Obsessive-Compulsive Scales-PDD (CYBOCS-ASD) The CYBOCS-ASD is a modified version of the CYBOCS developed for use in children with Obsessive-Compulsive Disorder (Scahill et al., 1997). The modified version is a semi-structured clinician-rated scale designed to rate the current severity of repetitive behavior in children with ASD (i.e., Time Spent, Interference, Distress, Resistance, and Control. Each item is scored from 0 (least symptomatic) to 4 (most symptomatic), yielding a Total score from 0 to 20. It has established reliability and validity (Scahill et al., 2006) and is sensitive to change (Scahill et al., 2014).

Caregiver Strain Questionnaire (CGSQ; Brannan, A.M., Heflinger, C., & Bickman, L, 1997) measures the impact of having a child with a ASD on the family. The questionnaire includes 21 items that assess three dimensions of caregiver strain: objective strain, internalized subjective strain, and externalized subjective strain. Each item is rated on a 5-point scale ranging from 1(not at all a problem) to 5 (very much a problem).

Parent Satisfaction Rating This will be developed for this study. Specific questions will be asked about parents assessment on the usefulness of specific techniques (e.g., the interview used to estimate the function of the behavior, the functional analysis, the use of placebo control). Parents will also be asked whether they would recommend the study to other parents and children with ASD and SIB.

Appendix B
HUMAN SUBJECTS
These inclusion and exclusion criteria described above will guide decisions on study entry.

Sources of Materials
The sources of the research material will be data collected in the course of the clinical trial to determine the efficacy and also to ensure the safety of study participants. The principal types of data will be clinical rating scales and standardized tests (e.g., IQ test). Vital signs and routine laboratory measures (blood chemistry, blood cell counts) and research blood sample will also be obtained.

Potential Risks
The risks associated with these procedures include exposure to the study drug or placebo, and the risk associated with venipuncture. The blood draws poses a possible risk of bruising at the site of the draw, light-headedness and fainting along with a remote chance of infection.

The safety record of NAC is excellent. When used as a dietary supplement, the dose of NAC ranges from 600 to 1000 mg/day. At the other extreme, NAC is given at very high loading doses (140 – 150 mg/kg in the first hour) by infusion in the treatment of acetaminophen overdose (Sandilands and Bateman, 2008). Although rare, anaphylactoid reactions consisting of angioedema, bronchospasm, tachycardia, flushing and hypotension have been reported with IV treatment. Zheng et al. (2014) used 600 mg of NAC twice a day in their 1 year study on the rate of COPD exacerbations compared to placebo. The study included 482 adults in the NAC and 482 adults in the placebo. The rate of serious adverse events (mostly consisting of COPD worsening) was nearly identical in the two groups. Other AEs included abdominal pain (2%), diarrhea (1%) and pruritus (1%).

Although highly unlikely, there are potential privacy risks associated with participating in such a study. There is minimal risk associated with the completion of rating scales and assessment measures.

Protection from Risks & Discomforts Blood draws are common procedures and will be performed by a certified phlebotomist. We will offer a numbing cream prior to the blood drawing procedure. We will systematically review adverse events at clinic visits and will be available by telephone. Parents will be given phone numbers of the principal investigator and study physician – who will be available after hours. The PI and study physician have access to the pharmacy after hours – if it is necessary to break the blind.

The proposed dose of NAC in this study is higher than when it is used as a dietary supplement. But it has been used safely in children and adults in this dose range (Hardan et al., 2012).

Confidentiality will be maintained throughout the project period. With the exception of tests that require date of birth, research records will not include names or contact information. Subjects will be identified only by their code numbers. A file with personal health information (e.g., names and addresses) will be kept separately in a locked file cabinet.

Blood Drawing & Vital signs Protocol: Children with ASD, especially those who engage in challenging behaviors such as self-injury, are at higher risk of having problems with medical procedures such as blood tests. To assist with the safety and success of the blood drawing procedure, we will follow these steps.

- At the initial screen appointment, we will discuss the blood drawing and blood pressure collection procedures with the caregiver and gather information about how these procedures are usually done with the child.
- If the caregiver has a history of successfully completing these procedures with the child, we will encourage the caregiver to follow the same strategies that have worked in the past. Caregivers will also be encouraged to stop

the procedure if they feel they child is demonstrating greater resistance or distress than has occurred in similar situations, or if they feel uncomfortable with the level of the child's distress.

- If the caregiver reports a history of unsuccessfully completing these procedures, we will review the options below to confirm the caregiver's approval. Note: these approaches will have been discussed in the consent process, but will be reviewed again in advance of the actual procedures.
    - Research staff will assist the caregiver in getting the child to the location for the procedure (i.e., nurses office or behavioral session room for vitals and an exam room at the Emory Children's Pediatric Center for the blood draw). This will be done by offering preferred items to the child in the location. A guided transition procedure will be used if necessary (described below) .
    - The child will be allowed to remain in the area where the procedure will be conducted with highly preferred items (e.g., toys, iPad) until the caregiver reports that the child is calm enough to attempt the procedure.
    - The clinician will attempt the procedure twice. If the child is resistant and the blood draw cannot be completed, the research staff will use physical guidance to keep the child safe while the clinician attempts the procedure up to two more times.
    - If the procedure is not successful after the above procedures, we will stop attempting and document that the procedure as unsuccessful due to noncompliance.
    - The caregiver will be present for all of these steps. As note, the caregiver will be allowed to stop the process in response to heightened distress level for the child (e.g., the child's distress is greater than exhibited in similar situations).
- If the blood draw or other vitals are not collected due to noncompliance, the research team (including study medical personnel) will meet and review all available information, including past information from the medical record if available through our HIPPA waiver. Based on this information, the team will make a decision about the child's eligibility for the study. The team conference may also include consultation with the child's primary care provider.

Transition procedure involves 1-2 staff members physically guiding the child from one location to another (or keeping the child in a specific safe location). This procedure is approved by the personal protective procedures program used in this patient population at the Marcus Autism Center. All staff members that use the personal protective procedures have undergone a 2-day training and passed both a written and a role-play examination to confirm they are capable of implementing the procedure safely.

Recruitment

All recruitment materials such as flyers, letters and social media posts will be IRB approved. Announcements will be placed on the Marcus website. We will send notices to primary care clinics, local parent organizations, and schools. We will also contact children and families on the Severe Behavior Clinic waiting list at Marcus.

Cost

There will be no costs for study visits of the study medication. The study medication and the matching placebo will be supplied by the BioAdvantex company.

Compensation

There will not be any compensation for study participation.

Consent Process

Interested families will be screened on the telephone by the study coordinator. Presumably eligible and willing families will be invited to Marcus for a formal screening visit. Parental consent will be obtained at the screening visit prior to the collection of any study data. The consent procedure will include the following: (a) provide the consent forms to parents and guardians together with a verbal summary of the contents of all paragraphs, and (b) a description of alternatives to participating in the study (not to take part, use of other treatments, and obtaining similar treatment through other

clinical services). Parents (guardians) will also be told that declining participation will not prejudice their right to pursue other treatments at Marcus, Children's Hospital or Emory.

Parental consent will be obtained by trained investigators as documented on the delegation of authority log. Children whose parents indicate notable barriers to meeting the demands of the study or whose parents lack capacity to understand the purpose and expectations of the study will not be randomized in the study. These judgments will be made by the investigator conducting the consent discussion in consultation with the research team as needed. Parents will be given the opportunity to ask questions. Once all questions have been answered, parent(s) will be asked to sign and date the consent form. Parents will be provided with a copy of the singed consent form.

Child Assent The children in this study (ages 5 to12 yrs 11 months) will be diagnosed with ASD and repetitive SIB. Some will also be cognitively delayed. The PI or designated Co-investigator conducting the consent procedure will evaluate the child's capacity to provide written or verbal assent. The investigator's judgment will be documented on the assent form.

Consent for Video Recording Consent for video recording for research purposes will be obtained from parents. If parents are willing, video recordings will be used for training purposes (e.g., presentations at professional meetings). Parents can indicate agreement or disagreement with this additional use of the videos directly on the video consent form. Agreement to use the videos for these additional training purposes is not required for study participation.

Study Withdrawal Parents are free to withdraw from the study at any time. This will be clearly stated during the consent process. Subjects may need to discontinue participation in the study before completion for various reasons (e.g., family move, withdrawal of consent, adverse events, need for a different treatment). Parents who indicate intention to withdraw the child from the study will be offered to have a case conference with the study team to discuss the matter. If the discussion indicates that the child needs another treatment, we will assist the family to locate that treatment. Parents who drop out of treatment will be invited to return for scheduled assessments or an early termination visit. Data collected from children who drop out of treatment, but return for assessments, will be analyzed in their originally randomized group.

Confidentiality

Case report forms (CRFs) contain the subject's unique ID number – but no identifying information. DEX is a secure, password protected data base. The folders containing CRFs data will be kept in locked files and access to these files is only granted to members of the research team. Standardized tests (e.g. IQ and language tests) record the child's date of birth in order to calculate the child's age for comparison to normative data. No other personal health information will be documented on research forms. Contact information for the family is kept in a separate file that is in a locked cabinet.

Certain measures will be recorded on video. The digital files are stored on a secure, password-protected site. Recordings may also be stored electronically on an encrypted external hard drive that is kept in a locked closet or cabinet. The digital files will be marked with a Subject ID instead of names of children or their parents. It is possible that names may be mentioned on the recordings. Video recordings from this research study will be retained for up to 5 years after the study is over. Recordings may be used for other purposes (e.g. presentations at professional meetings) if separate consent is obtained from the parent (or guardian).

Access to study records (case report forms) will be restricted to study staff. Others that have access to the record include individuals with regulatory responsibility at Emory, Children's Health Care of Atlanta (CHOA). All clinical trials are subject to routine audits by offices at Emory or CHOA.

Potential Benefits of the Proposed Research to the Subjects and Others

Subjects may directly benefit by the receipt of NAC. Subjects initially randomized to placebo will be offered treatment with NAC in the 9-week, open-label extension trial. Overall, the risks to the subjects in this research are similar to those that exist in standard clinical practice and the proposed research project offers the potential for improving treatment of

moderate repetitive behavior in children with ASD. Indeed, this research may lead to a new treatment option for repetitive behavior in children with ASD. Thus, the potential benefits of the research outweigh the risks involved.

Importance of the Knowledge To Be Gained Children with ASD with language delay are impaired and, untreated, their long-term prognosis is guarded. Although current treatments are helpful in some cases, significant gaps in our treatment options remain. There are no large, controlled clinical trials of NAC in children with ASD and no medication treatments for the core feature of repetitive behavior in children with ASD. This study may provide new information about the treatment of repetitive behavior in children with ASD and may identify subgroups of affected children who could benefit from this treatment.

Data Safety and Monitoring Plan
This pilot study will not have an external Data and Safety Monitoring Board. It will be conducted in accordance with "good clinical practice" as outlined in the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (Dixon & Hallinan, 1999).

The Steering Committee will be comprised of the PI, Drs. Call, Scheithauer and Sidhu, Mr. Gillespie and the data manager from DEX. Drs. Scahill and Sidhu and the data manager will meet at least once a month to discuss the conduct of the trial including recruitment, attrition, attendance and rate of successful data collection. Problems or concerns identified will be reviewed with the Steering Committee will provide oversight and ensure that the study is conducted in accordance with the protocol.

The unblinded data manager (member of the DEX team) will serve as an independent monitor of data quality and safety with input from Dr. Scahill as needed. The data manager will have direct access to the study data base in DEX and will conduct regular checks on recruitment, attendance, missing data, attrition and adverse events. Mr. Gillepsie will provide a summary every six months for Dr. Scahill's review of these same metrics (recruitment, attendance, missing data, attrition and adverse events). Questions and concerns will be brought to the Steering Committee as needed. To protect the blind, treatment assignment will not be divulged. The discussion with the Steering Committee is likely to focus on individual cases and overall trends. Given the sample size of 14 (7 per group) in this pilot study, we have not declared any **stopping rules** for benefit or futility. In the unlikely occurrence of a serious adverse event, Dr. Scahill will confer with Dr. Sidhu (Medical Director at Marcus) to judge whether the event is an SAE and whether it is possibly, probably or definitely related to the study drug (see below for reporting SAEs). This review of potential SAEs will include deliberation on whether to break the treatment blind for the participant. In each situation, we will presume that the participant is on the active medication and consider the possible medical implications – given that assumption. The blind will be broken only if medically indicated by the PI and Dr. Sidhu. The treatment assignment will not be disclosed to the treating clinician, independent evaluator, coordinator, or behavior data analysts.

Monitoring for Adverse Events (AEs):
At each visit, including baseline and interim contacts, the treating clinician will systematically review for adverse effects and concomitant medications using an Adverse Effects Review form developed for this study. We have used versions of this form, in several multi-site clinical trials in children with autism (RUPP Autism Network, 2002; Autism Network, 2005b; Scahill et al., 2006; Aman et al., 2009; Scahill et al., 2015). It contains a general inquiry, drug-specific queries, and several questions about daily activities (e.g., sleep, appetite, energy level, bowel and bladder functions). The general inquiry includes an open-ended question about any problems or complaints, as well as questions regarding the need for other medications and doctor or health care encounters since the last study visit. The next section includes drug-specific queries, probing for adverse events that have been reported in the scientific literature. These items are rated as not present, mild, moderate or severe. The ratings: mild, moderate or severe are defined as follows: mild= present, but no intervention required; moderate=present, may be bothersome or may require intervention; severe=present, bothersome and requires intervention. The last section includes specific questions about daily activities. All **new** adverse events (mild, moderate or severe) will be documented on the adverse event log. The status of previously-reported adverse events will be monitored as well. In the prior RUPP Autism Network studies, the form was easily modified to

make it relevant for risperidone (RUPP Autism Network, 2002), methylphenidate (RUPP Autism Network, 2005b) and guanfacine (Scahill wet al., 2015). We will include queries about self-harm or expressions of suicidal thoughts or plan.

When the treating clinician elicits an adverse event (AE), it will be documented regardless of suspected relationship to the study drug. The adverse event log requires the treating clinician to label the AE (using a list of preferred terms and a numeric code) and to document the severity, onset, course, outcome, and attribution (study drug related or not).
Attribution is classified as follows:

- Definite: AE is clearly related to the study drug.
- Probable: AE is likely to be related to the study drug.
- Possible: AE may be related to the study drug.
- Unlikely: AE is doubtfully related to the study drug.
- Unrelated: AE is clearly not related to the study drug.

Severity of AEs is classified as follows:

- Grade 1 mild= present, clinical observation only, intervention not indicated;
- Grade 2 moderate = present, minimal, local or noninvasive intervention indicated; may limit age-appropriate instrumental activities of daily living;
- Grade 3 severe = present, medically significant but not immediately life-threatening; may not need hospitalization or may not prolong current of hospitalization indicated; potentially disabling; likely to limit self-care activities of daily living. NOTE: if hospitalization is required – prepare Serious Adverse Event report (see below).
- Grade 4 Life-threatening (serious adverse event) = urgent intervention indicated.
- Grade 5 = Death.

During the study, participant with Grade 3 or 4 AE should receive appropriate treatment and may have to stop study drug. Decisions on the appropriate care of the subject will be made by the treating clinician, the PI, the research study team (all of whom will remain blind to study treatment). The treatment plan will be set up in collaboration with the child's primary care provider whenever possible. Depending on the nature of the Grade 3 or 4 AE, the research team, in consultation with the family, may recommend unscheduled medical testing. Abnormal findings on laboratory tests will be investigated to inform the treatment plan. If the abnormality fails to normalize after stopping study drug, the subject should be referred and followed until resolution of symptoms.

Routine reporting of AEs Adverse events will be documented on the Adverse Event Log as described above. All AE data will be captured in the electronic database and reviewed by Drs. Scahill and Sidhu on a regular basis, at least quarterly. AEs occurring at a greater than expected frequency or severity will be reviewed with the Steering Committee. Investigators will provide this information to the Emory IRB based on IRB Policy & Procedures (P&Ps). The Steering Committee will decide if modifications to the protocol or consent form are required.
AEs resulting in treatment discontinuation: Adverse events that are the primary reason for study discontinuation will be tracked separately. The data manager will provide a table with all such side effects to the PI three times a year.
Serious adverse events: A serious adverse event (SAE) is defined as an event that poses a threat to the participant's life or functioning. We note that "severe" is not necessarily equivalent to "serious." Thus, a severe rash may not be a serious adverse event, whereas a heart attack of any severity is likely to be a serious adverse event. A serious adverse event is defined as any event that entails one of the following:

- Death;
- Threat to the individual's life;
- Inpatient hospitalization or prolongation of existing hospitalization;
- Persistent or significant disability or incapacity;
- Intentional drug overdose;
- Any other significant event that jeopardizes the participant.

Assessment and Reporting of Serious Adverse Events If an adverse event meets the definition of an SAE, the PI in consultation with Dr. Sidhu will determine whether the SAE is possibly, probably of definitely related to the study drug.

The PI will report SAEs to the local IRB as required by IRB P&Ps (within10 business days) under the following circumstances: a) serious AND unanticipated AND possibly, probably or definitely related events; and b) anticipated adverse events occurring with a greater frequency than expected. SAEs that do not meet any of these circumstances will be reported with IRB re-approvals. Written IND safety reports will be submitted to the FDA by the IND sponsor, Dr. Scahill, for serious, unexpected suspected adverse reactions within 15 calendar days of learning of its occurrence. If the event is fatal or is deemed to be life threatening, the report will be made within 7 calendar days. The IND sponsor will also make an assessment of whether the event constitutes an unanticipated problem (UP) posing risks to subjects or others. This assessment will be provided to the Emory University IRB, which, in turn will make a final determination. If the Emory IRB determines an event is a UP, it will notify the appropriate regulatory agencies and institutional officials.