Study Title: A phase II, randomized, double-blind, placebo-controlled study of myrosinase-enriched glucoraphanin, a sulforaphane precursor system, in autism spectrum disorder

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A phase II, randomized, double-blind, placebo-controlled study of myrosinase-enriched glucoraphanin, a sulforaphane precursor system, in autism spectrum disorder

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List of Abbreviations

ABC- Aberrant Behavior Checklist
ADOS-2- Autism Diagnostic Observation Schedule, 2nd edition
AE- adverse event
Akt- protein kinase B
ALT- alanine aminotransferase
ARE- Antioxidant response elements
ASD- autism spectrum disorder
AST- aspartate transaminase
ATP- adenosine triphosphate
BOM- behavioral outcome measures
BSE- Broccoli Sprout Extract
CBC- complete blood count
CGI- Clinical Global Impression Scale
CIDD- Carolina Institute for Developmental Disabilities
CMP- comprehensive metabolic panel
CNS- central nervous system
CRF- case report form
DNA- deoxyribonucleic acid
DSM-5- Diagnostic and Statistical Manual, 5th edition
DSMB- Data and Safety Monitoring Board
DTC- dithiocarbamates
EEG- electroencephalogram
FDA- Food and Drug Administration
GLMM- general linear mixed models
GMP- Good Manufacturing Practice
GR- glucoraphanin
GS- glucosinolate
GSH- reduced glutathione
GSSG- oxidized glutathione
GST- glutathione-S-transferase
HIPAA- Health Insurance Portability and Accounting Act
HO-1- heme oxygenase-1
HSF1- heat-shock transcription factor 1
HSP- heat shock protein
IDS- Investigational Drug Services
IF-γ- interferon-gamma
IL-6- interleukin-6
iNOS- inducible nitric oxide synthase
IRB- internal review board
ITC- isothiocyanate
IQ- intelligence quotientswi
Keap1- Kelch-like ECH-associated protein
LAR- legally authorized representative
LFTs- liver function tests
LPS- lipopolysaccharide
mTOR- mammalian target of rapamycin

NC TraCS- North Carolina Translational and Clinical Science Institute
NF-KB- nuclear factor-KB
Nrf2- nuclear factor- erythroid factor 2 (Nrf2)
NSAID- non-steroidal anti-inflammatory drug
OACIS- Autism Clinical Impressions Severity and Improvement Scales
OTU- Operational Taxonomic Unit
PHI- Protected health information
PI- Principle Investigator
PI3K- phosphoinositide-3 kinase
PBMC- peripheral blood mononuclear cell
PSA- prostate specific antigen
PTEN- phosphatase and tensin homolog
RBSR- Repetitive Behavior Scale-Revised
RCT- randomized controlled trial
ROS- reactive oxygen species
RTI- Research Triangle Institute
SAE- serious adverse event
SB-5- Stanford-Binet Intelligence Scales, 5th edition
SF- sulforaphane
SRS- Social Responsiveness Scale
TNF-α- tissue necrosis factor-alpha
TSC1/2- tuberous sclerosis complex
TSH- thyroid stimulating hormone
T3- triiodothyronine
T4- thyroxine
UNC- University of North Carolina

Study Summary

Title	A phase II, randomized, double-blind, placebo-controlled study of myrosinase-enriched glucoraphanin, a sulforaphane precursor system, in autism spectrum disorder
Short Title	Sulforaphane in autism spectrum disorder
Protocol Number	C15-0560
Phase	Phase II
Methodology	Randomized, double-blind, placebo-controlled trial
Study Duration	24 months
Study Center(s)	Single-center: University of North Carolina- Chapel Hill (Carolina Institute for Developmental Disabilities)
Objectives	(1) To determine if a sulforaphane supplement (Avmacol®) is effective for improving social impairment in autism spectrum disorder (ASD); (2) To determine if Avmacol® is safe and well-tolerated in adolescents and young men with ASD. (3) To explore if Avmacol® improves behavioral symptoms commonly associated with ASD.
Number of Subjects	54 subjects
Diagnosis and Main Inclusion Criteria	Healthy males between ages 13-30 years old with a diagnosis of moderate to severe ASD
Study Product, Dose, Route, Regimen	Avmacol® uncoated tablets, each containing 125 mg broccoli seed extract (source of glucoraphanin) and 50 mg broccoli sprout extract (source of myrosinase) or matched placebo tablets; 3-8 tablets by mouth once daily (specific dose determined by weight).
Duration of administration	12 weeks
Reference therapy	Matched placebo tablets
Statistical Methodology	General linear mixed models will be used to test the primary and secondary hypotheses; of interest will be the main effect of treatment, treatment x time, and treatment x time x treatment phase. Analyses will include data from all randomized subjects who received at least one dose of study medication and participated in at least one post-baseline efficacy assessment.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations, and University of North Carolina research policies and procedures.

1.1 Background

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by severe and persistent deficits in social communication and restricted, repetitive behaviors and interests [1]. ASD is now estimated to affect 1 in 68 children in the U.S., including 1 in 42 boys [2], and leads to substantial, lifelong burden for individuals, families, and communities. Use of psychiatric medication in ASD is high, with 66% of affected individuals taking at least 1 psychotropic medication by adolescence [3]. However, no medications are currently available to treat the defining clinical features of autism, and pharmacological management is instead limited to treating associated behavioral symptoms, such as hyperactivity, self-injury, and aggression. Only risperidone and aripiprazole have FDA approval for use in ASD, specifically for the treatment of severe irritability in children [4]. Faced with heavy side effect burden and often limited efficacy, patients and their families frequently turn to supplements and alternative treatments that promise low toxicity but lack rigorous investigation and oversight.

Developing interventions specifically for ASD has been hindered by heterogeneity in its phenotypic expression and implicated genes, epigenetic effects, and molecular pathways; as such, no treatments based on an understanding of disease mechanism are presently available. However, several emerging lines of research suggest that interrelated metabolic dysfunction and immune dysregulation at the cellular level contribute to the pathogenesis of ASD in at least a subset of individuals [5, 6]. Preclinical and human studies have documented increased levels of oxidative stress [7-9]; reduced antioxidant activity [10-12]; mitochondrial dysfunction [13-15]; elevated inflammatory markers [16-19]; evidence of neuroinflammation [20-23]; and synaptic dysfunction [24] in association with ASD. Evidence of cellular dysfunction provides an intriguing hypothesis for the systemic phenomena commonly observed in patients with ASD, including gastrointestinal problems and improvement in ASD symptoms during fever [25, 26]. Anecdotal improvement in core symptoms of autism during fever, particularly repetitive speech and behavior, is commonly observed, possibly as a result of upregulation of heat shock proteins (HSPs) that play a key role in multiple CNS cellular processes, including synaptic transmission and response to metabolic stress [25, 27, 28].

It is hypothesized that this varied evidence of cellular dysfunction may reflect impaired activity in common metabolic pathways, the consequence of which is aberrant protein synthesis that impedes normal synaptic development and function. Synaptic dysfunction is believed to be a key pathogenic mechanism in ASD [24]. In particular, overactivation of the mammalian target of rapamycin (mTOR) intracellular signaling pathway has been implicated in excessive protein synthesis and synaptic dysfunction in ASD [29-31].

The Kelch-like ECH-associated protein 1 (Keap1)/nuclear factor-erythroid factor 2 (Nrf2)/antioxidant response elements (ARE) pathway is a highly efficient system involved in maintaining cellular redox balance and reducing oxidative damage through a series of antioxidant molecules and detoxifying enzymes. Nrf2 is a nuclear transcription factor that, when activated by oxidative or electrophilic conditions, translocates from the cytoplasm to the nucleus and induces expression of a variety of antioxidants and immune mediators involved in limiting cellular damage from these conditions [32, 33]. ASD diagnosis in children has been linked to a 45% reduction in gene expression of Nrf2 in association with evidence of greater oxidative stress [6]. These findings suggest that deficits in the Keap1/Nrf2/ARE pathway may lead to a state of chronic inflammation and decreased capacity to respond effectively to conditions promoting oxidative stress in autism. While it is not yet clear if these cellular abnormalities are etiological or secondary manifestations of another disease-causing mechanism, preliminary studies have suggested that treatments aimed at reducing oxidative stress and mitochondrial dysfunction may improve core and associated symptoms of ASD [34]. In keeping with this theory, compounds that upregulate Nrf2 activity may have the potential to improve symptom burden in ASD.

Sulforaphane (SF) is a naturally-occurring isothiocyanate (ITC) in cruciferous vegetables that induces HSP and Nrf2 activity, leading to enhanced redox homeostasis, DNA repair, and fatty acid and lipid cellular metabolism [32]. SF has also been shown to (1) suppress lipopolysaccharide (LPS)-induced inflammation in rat microglia [35], (2) reduce neuroinflammation in degenerative CNS disorders [36], and (3) mitigate reactive oxygen species (ROS)-mediated neurotoxicity in a murine model of herpes encephalitis [37] through activation of Nrf2. In cultured cardiomyoctes and prostate cancer cells, SF reduces cellular damage through downregulation of mTOR-mediated excessive protein synthesis [38, 39].

Due to its chemoprotective properties, SF has been investigated as a therapeutic agent in clinical trials of prostate cancer, breast cancer, exposure to air pollutants, and insulin resistance in type 2 diabetes with promising results [40-44]. Importantly, SF is bioavailable when taken orally and readily crosses the blood-brain barrier [45-47]. SF is attractive as a potential therapeutic compound in ASD for its demonstrated capacity to improve redox balance, enhance antioxidant capacity, reduce neuroinflammation, support cellular metabolism, and regulate the mTOR signaling pathway, all of which have been implicated in autism pathogenesis. In a recent clinical trial, Singh and colleagues [48] first demonstrated that young men with autism treated with oral SF for 18 weeks were rated as calmer by caregivers and showed improvement in standardized measures of social communication compared with a placebo group. In this proposed clinical trial, we aim to independently replicate this important study and add to the evidence base of SF treatment for autism.

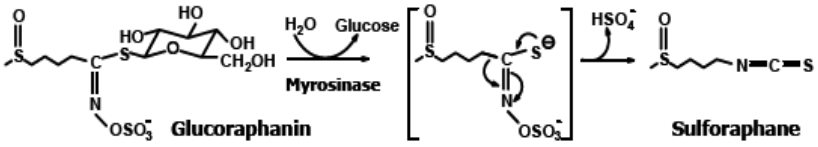
Of note, the trial by Singh et al [48] utilized freeze-dried, SF-rich broccoli sprout extracts, an investigational product formulated by the Cullman Chemoprotection Center at Johns Hopkins University specifically for use in clinical trials. Hydrolyzed Broccoli Sprout Extract (BSE) is costly, difficult to manufacture, and no longer produced in the United States for clinical trials or routine clinical use. Avmacol® is a commercially available nutraceutical that provides a rich source of SF through co-delivery of glucoraphanin (GR), a sulforaphane precursor, and myrosinase, the plant enzyme that catalyzes conversion of GR to SF. Avmacol® is readily available as a dietary

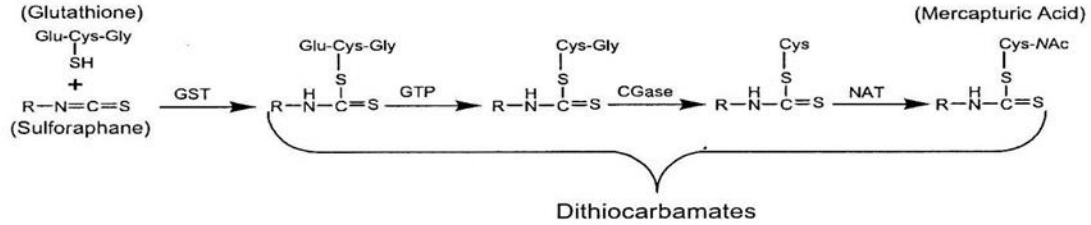
supplement, is shelf-stable at room temperature, and offers the potential for post-study continuance of therapy. The proposed study will explore if this SF precursor-enzyme product confers similar therapeutic benefit for core deficits in ASD as freeze-dried BSE, which will have important broad implications for treatment.

1.2 Investigational Agent

The investigational product, Avmacol® (Nutramax Laboratories, Inc., Edgewood, MD) is a dietary supplement containing broccoli seed extract and freeze-dried broccoli sprouts that is manufactured under GMP standards and has been commercially available since 2013. Avmacol® provides a rich source of the isothiocyanate (ITC) sulforaphane (1-isothiocyanato-(4R)-(methylsulfinyl)butane) in the form of glucoraphanin (4-methylsulfinylbutyl-glucosinolate), an inert glucosinolate (GS), and myrosinase, the plant enzyme that hydrolyzes glucoraphanin to sulforaphane [49]. The chemoprotective properties of cruciferous vegetables (broccoli, cauliflower, cabbage, etc.) are largely attributed to their high content of GS, which are converted to biologically active ITCs (i.e., sulforaphane) by myrosinase and by the microflora of the gastrointestinal tract after ingestion [50]. Myrosinase is present in plant cells and is physically segregated from the glucosinolates until the barrier is ruptured via cell damage or chewing.

SF is rapidly conjugated with glutathione by glutathione-S-transferases (GSTs) [51, 52] and accumulates in various types of cells through conjugation with cellular glutathione [53, 54]. SF conjugates are exported from the cell by a transporter-mediated mechanism [54]. Successive steps of hydrolysis of the conjugates lead ultimately to formation of N-acetyl-cysteine derivatives (mercapturic acids). These conjugates are dithiocarbamates (DTC) that are excreted in urine and can be quantified through a cyclocondensation reaction developed in the Cullman Chemoprotection Center of Johns Hopkins University. SF activates the Nrf2 pathway, resulting in elevated gene transcription via the Antioxidant Response Element (ARE) in the regulatory domain of its target genes [55, 56].





Each tablet of Avmacol® contains 125 mg broccoli seed powder and 50 mg broccoli sprout extract, providing approximately 15 mg (34.4 µmol) of glucoraphanin and enough myrosinase to fully hydrolyze the glucoraphanin contained in the tablet. In the batch of tablets formulated for the proposed clinical trial, manufacturing specifications required at least 13 mg glucoraphanin per tablet, and recent analysis indicated 17 mg per tablet (see Avmacol® COA in Section 7). Manufacturing data from Nutramax, Inc. and bioavailability studies of similar compounds containing GR and myrosinase suggest a bioconversion to SF rate in the range of 40-50% [47]; it is expected that each Avmacol® tablet with therefore supply approximately 15 µmol SF following bioconversion. Exact amounts of bioavailable SF will varies somewhat from individual to individual depending upon endogenous enzymatic conversion following ingestion.

Healthy human volunteers ingesting 200 µmol of SF from a broccoli sprout extract preparation demonstrated rapid absorption, first-order metabolism, and urinary excretion of metabolites (DTCs), yielding a mean half-life of 1.77 ± 0.13 hr. and volume of distribution of 59.9 ± 7.0 liters [57]. Despite the relatively short half-life, SF is believed to have a longer duration of action, as induced phase 2 enzymes have half-lives measured in days [56]. SF has been shown to cross the blood-brain barrier [58]. Urinary metabolites (DTCs) are renally excreted and can be quantified using a cyclocondensation reaction.

1.3 Preclinical Data

A number of animal studies have investigated the biological effects and toxicology of oral broccoli sprouts and SF, demonstrating overall *in vivo* safety and beneficial biological effects, namely via induction of phase 2 antioxidant enzymes, which have chemoprotective properties. Rodents treated with relatively high doses of SF and broccoli sprout extracts have not demonstrated any significant change in body weight or evidence of organ toxicity [59-62]. SF has also shown little cytotoxicity *in vitro*, though hepatotoxity in rats has been demonstrated at extremely high concentrations (>500 µM) [63].

Numerous studies have associated ASD with cellular abnormalities, including: (1) redox imbalance and oxidative stress [9, 10, 64]; (2) mitochondrial dysfunction [13-15]; (3) immune dysregulation/ neuroinflammation [20-23, 65]; (4) symptomatic improvement with fever/ heat shock response [25, 27]; and (4) aberrant mTOR expression and its effects on synaptic transmission [29-31]. It is hypothesized that these varying types of cellular dysfunction may involve a number of related, interacting metabolic pathways [11], though the relationship between them has not been fully characterized. Preclinical data has identified a role for SF and its ability to upregulate the Keap 1/Nrf2/ARE pathway in modulating each of these areas of cellular dysfunction, suggesting SF could have therapeutic benefit in autism.

Upregulation of antioxidant enzymes via activation of the Keap 1/Nrf2/ARE pathway has important implications for ASD, which has been associated with reduced levels of Nrf2 expression and oxidative phosphorylation capacity in cultured cells [6]. Lower concentrations of reduced

glutathione (GSH), the primary intracellular antioxidant, higher levels of oxidized glutathione (GSSG), and reduced GSH/GSSG redox ratios have also been identified in plasma, peripheral blood mononuclear cells (PBMCs), lymphoblastoid cell lines, brain tissue, and mitochondria of individuals with autism in comparison to healthy controls [9, 64, 66, 67]. SF has been found to have neuroprotective effects against oxidative stress through Nrf2 induction and the resulting increased expression of antioxidant enzymes and elevation of GSH [68-73]. In a rat model of spinal cord contusion, SF readily crossed the blood-brain barrier, increasing phase 2 enzyme expression and reducing inflammation at the site of the injury [74]. Similarly, in a murine model of herpes encephalitis, SF stimulated Nrf2 activity and antioxidant enzyme (heme oxygenase-1, glutathione peroxidase 1) expression in astrocytes, inhibiting ROS-mediated neurotoxicity [37]. Animal models of neurodegenerative diseases suggest that SF can have a neuroprotective effect by limiting damage from oxidative stress and inflammation [36, 75].

Evidence of mitochondrial dysfunction in individuals with ASD has been demonstrated in brain mass resonance spectroscopy imaging [76], cell lines derived from children with ASD [14], and in blood cell [13, 77] and brain samples [15] of children with ASD, and this dysfunction may play a critical role in disease pathogenesis in a subset of individuals [11, 78, 79]. In particular, impairment in fatty acid oxidation is believed to be one of the major contributors to the impaired mitochondrial phenotype [80], supported by elevated levels of acyl-carnitines [81] and decreased levels of free carnitine [82] in some individuals with ASD. Emerging evidence suggests that Nrf2 plays a role in supporting mitochondrial function and metabolism, possibly through improving the efficiency of fatty acid oxidation [80, 83]. In a rodent hippocampal model, SF was associated with increased adenosine triphosphate (ATP) synthesis and decreased vulnerability to induced seizures [84]. SF also induced mitochondrial and peroxisome biogenesis in cultured fibroblasts [85].

Inflammation and immune dysregulation has been well-documented in animal models and individuals with ASD, both peripherally and within the central nervous system [5, 11, 22, 65]. ASD is associated with elevated rates of microglial activation in murine models, post-mortem studies, and brain imaging [20, 21, 23, 86] as well as heightened expression of proinflammatory cytokines in the CNS, particularly interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IF-γ) [16-19]. SF has been shown to modulate neuroinflammation in animal models [35, 37, 74]. The beneficial effect of SF on inflammation is mediated at least in part through inhibition of the nuclear factor-KB (NF- KB) pathway, resulting in decreased expression of numerous proinflammatory factors [80]. Nrf2-deficient mice demonstrate increased microglial activation and release of proinflammatory cytokines in response to LPS challenge [87]. When treated with SF, mice exposed to LPS show attenuated neuroinflammation, as evidenced by reduction in microglial production of the proinflammatory cytokines inducible nitric oxide synthase (iNOS), IL-6, and TNF-α [87].

Common anecdotal reports and observational studies suggest that some individuals with ASD experience a substantial but temporary improvement in autistic symptoms (primarily stereotypic behavior and in appropriate speech) during fever [25], unrelated to the severity of fever or autism. Though the mechanism of this "fever effect" is not entirely clear, direct thermal effects as well as indirect effects, including increased expression of heat shock proteins (HSPs) and activation of cellular stress responses, may be involved [85, 88]. HSPs have a cytoprotective effect during

thermal stress, functioning as molecular chaperones for misfolded proteins and playing a role in cellular repair [88]. In addition, HSPs are central to synaptic transmission and may improve long-range cerebral cortical connectivity that is depressed in ASD [80, 89]. SF has extensive effects on HSPs [85, 90], including induction of the HSP heme oxygenase 1 (HO-1, or Hsp32) [91], activation of heat-shock transcription factor 1 (HSF1), and upregulation of Hsp27, which increases proteasomal activity [85]. SF may therefore ameliorate features of autism, in part, by inducing expression of cytoprotective HSPs [80].

Finally, dysfunctional synaptic transmission caused by aberrant protein synthesis is believed to play a key pathogenic role in ASD [24], potentially due to overactivation of the phosphoinositide-3 kinase/protein kinase-B/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling pathway [31, 92]. Mutations in genes upstream of mTOR (e.g., phosphatase and tensin homolog (PTEN), tuberous sclerosis complex (TSC1/2)) lead to overactivity of the mTOR pathway, resulting in excessive protein synthesis, abnormal synaptic function, and ASD phenotypes [80]. Synaptic plasticity requires a balance between protein synthesis and degradation [93], and deficits in autophagy, a major protein degradation pathway downstream of mTOR signaling, are also associated with overactivation of the mTOR pathway and ASD dendritic spine pathology [31]. SF has been shown to downregulate mTOR signaling and associated excessive protein synthesis in breast cancer [94], cardiomyocyte [38], and prostate cancer cell lines [39], as well as induce autophagy in neuronal cells and the brains of mouse models for Huntington's disease [95, 96]. mTOR signaling and protein synthesis are also inhibited in acute lymphoblastic leukemia cells treated with SF [97]. Taken together, these findings suggest that SF modulates aberrant mTOR signaling activity and autophagy, which may have a therapeutic effect for ASD.

Clinical Data to Date

Sulforaphane (SF), derived from freeze-dried broccoli sprouts, was recently investigated as a treatment for symptoms of ASD in a randomized, double-blind, placebo-controlled trial of 45 male adolescents and young adults (ages 13-30 years old) with moderate to severe autism (clinical trials identifier NCT01474993) [48]. Fifteen participants were randomized to placebo and 29 to SF; each group was treated for 18 weeks, followed by a 4-week discontinuation period and final follow-up at 22 weeks. Forty participants (14 on placebo, 26 on SF) completed the trial; of those participants, 32 (80%) had a reported history of behavioral improvement during fever episodes. The primary outcome was change in symptoms of autism as measured by change in Social Responsiveness Scale (SRS) total scores; additional measures of interest included change in caregiver-completed Aberrant Behavior Checklist (ABC) scores and clinician-rated Autism Clinical Impressions Severity and Improvement Scales (OACIS-Severity and OACIS-Improvement). SRS scores improved significantly more in the SF group compared with the placebo group (-15 units vs -3 units; p=0.02). Considering the 4 non-completers as non-responders, 31% (9/29) SF participants vs 0% placebo participants demonstrated a 25% decrease in total SRS score from baseline (p=0.018). SF participants also demonstrated significantly greater improvement in ABC total and OACIS-Improvement scores, particularly in the domains of social interaction (p=0.003), aberrant/ abnormal behavior (p=0.007), repetitive/ stereotypical behavior (p=0.08), and verbal communication (p=0.008). SF was well-tolerated with no serious adverse events. Two subjects

with a history of seizures had single unprovoked seizures during the course of the study (one after 3 weeks on SF, one 3 weeks after discontinuing SF), and the possibility of seizure as potentially associated with SF could not be ruled out.

Based on available data from Phase I human clinical trials of SF-rich compounds, SF appears to be extremely well-tolerated with minimal risk aside from mild gastrointestinal symptoms, which may include bloating, nausea, and loose stools.

A Phase I study of orally administered SF- and GR-containing BSE (75 µmol glucosinolates, 300 µmol glucosinolates, or 75 µmol isothiocyanates daily) for 7 days in 12 healthy volunteers did not demonstrate any clinical adverse events [51]. Two of 12 subjects assigned to active treatment demonstrated an increase in plasma ALT exceeding the upper limit of normal, though ALT levels rose for all subjects during the course of the study (including those assigned to placebo control), and the increase was not clearly attributable to consumption of BSE. Three of 12 subjects (2 active treatment, 1 placebo) demonstrated TSH levels exceeding the upper limit of normal during or after the dosing period, though elevated TSH was not associated with clinical symptoms or T3/T4 abnormalities.

Egner et al. [46] conducted a cross-over trial in 50 participants, randomized to ingest a SF-rich beverage (150 µmol/day) for 7 days in a first or second phase. Two of 50 participants complained of bitter taste or nausea, and no other adverse events were reported. No abnormal serum chemistry values were reported after 7 days. In a study of healthy participants ingesting a beverage containing 600 µmol of glucoraphanin and 40 µmol of sulforaphane, the intervention was well-tolerated with no laboratory evidence of effect on liver and kidney function [43].

A study of orally administered broccoli sprout homogenates (25 to 300 g) in 57 subjects over 3 separate days found no adverse effects aside from mild digestive effects [98]. Similarly, in a RCT of 20 men with recurrent prostate cancer treated with sulforaphane 200 µmol/ day for 20 weeks, no serious adverse events occurred, though some reported mild gastrointestinal upset [40].

A randomized, placebo-controlled trial of the sulforaphane product Prostaphane® in 81 men with rising prostate specific antigen (PSA) values following radical prostatectomy for prostate cancer represents the highest daily dosing of sulforaphane to date (338 µmol) [99]. No serious adverse events were reported, though gastrointestinal bloating was experienced somewhat more often in the SF group (N=17 vs. N=10), and one participant in the SF group withdrew from the study due to bowel discomfort. The differences in adverse effects between the two groups were not statistically significant (p=0.149). Furthermore, no significant between-group differences were found for body weight, BMI, blood counts, and chemistries.

1.4 Dose Rationale and Risk/Benefits

The Avmacol® dosing regimen for the current study was designed to deliver a slightly higher concentration of SF as that administered in a previously reported clinical trial of SF for ASD [48]. In the randomized, placebo-controlled trial by Singh et. al [48], participants randomly assigned to the active treatment group received capsules containing SF-rich broccoli sprout extracts that provided approximately 50 µmol SF/cap. Dosing was weight-based as follows:

50 µmol SF (1 cap) daily for weight < 100
100 µmol SF (2 caps) daily for weight 101-199 lb.
150 µmol SF (3 caps) daily for weight ≥ 200 lb.

Using this dosing schedule, participants receiving SF demonstrated significant improvement in social impairment as measured by change in Social Responsiveness Scale (SRS) total scores and significant improvement in related challenging behaviors as measured by the Aberrant Behavior Checklist (ABC) as compared with placebo over the course of the 16-week active treatment period. Between group differences were first observed by week 4 of treatment. These doses of SF were well-tolerated by participants and were not significantly associated with adverse events, change in blood pressure or heart rate, or change in routine laboratory values. Unexpectedly, SF treatment was associated with minor but significant weight gain, and 2 participants with a history of seizures had single, unprovoked seizures (one taking SF, one taking placebo).

The currently proposed clinical trial is designed to independently replicate the study by Singh and colleagues [48], to support or refute these findings. Avmacol® delivers SF in the form of its glucosinolate precursor, glucoraphanin (GR; derived by broccoli seed extract), and the active enzyme myrosinase (derived from broccoli sprouts), which hydrolyzes GR to SF upon ingestion. Bioavailability studies have demonstrated approximately 40-50% endogenous conversion of GR to SF upon ingestion of compounds containing GR enriched with myrosinase in healthy participants [47]. Each tablet of Avmacol®, formulated for use in clinical trials, contains approximately 15 mg (34.4 µmol) of glucoraphanin. Assuming 45% endogenous conversion of GR to SF (which will vary somewhat from individual to individual), each tablet will therefore supply approximately 15.5 µmol SF/tablet. The weight-based Avmacol®/ placebo dosing schedule for the current study is as follows:

3 tablets (approx. 46.5 µmol SF) if <100 lb
5 tablets (approx. 77.5 µmol SF) if 100-125 lb
6 tablets (approx. 93 µmol SF) if 126-175 lb
7 tablets (approx. 108.5 µmol SF) if 176-199 lb
8 tablets (approx. 124 µmol SF) if ≥ 200 lb

Based on previous studies of SF in human subjects, this dosing regimen is expected to be very well-tolerated with minimal to no adverse effects. This dosing regimen is also expected to deliver a slightly higher weight-based dose of SF than the trial conducted by Singh et al [48] after accounting for the 70% bioconversion rate (approximate) when purified SF is administered orally (i.e., an oral dose of 50 µg SF derived from freeze-dried BSE is expected to provide 35 µg SF following ingestion and bioconversion).

If a participant experiences significant adverse events that affect tolerability, may reasonably be related to taking the investigational product, and do not meet the protocol definition for a serious AE, the dose may be reduced by approximately 30% according to the following dose modification schedule:

Weight category	Prescribed weight-based dose of glucoraphanin	Prescribed # of tablets	70% of weight-based dose (ie, 30% reduction)	Prescribed # tablets for reduced dose (rounded to nearest 15 mg increment)*
<100 lb	45 mg	3	31.5 mg	2
100-125 lb	75 mg	5	52.5 mg	3
126-175 lb	90 mg	6	63 mg	4
176-199 lb	105 mg	7	73.5 mg	5
≥ 200 lb	120 mg	8	84 mg	6

**Rounded down if equidistant between two 15 mg dosing increments*

If the dose is reduced during the course of the study, the participant will continue the lowered dose for the remainder of the study. If adverse events do not improve within 14 days of lowering the dose, the investigational product may be permanently discontinued at the discretion of the treating study physician, participant, and participant's guardian. If a participant exhibits extreme exacerbation of behavioral symptoms (such as self-injurious behavior or aggression) that do not improve substantially following a dose reduction and are determined "probably" or "definitely" related to taking the investigational product, the investigational product may be permanently discontinued sooner than 14 days post-reduction, and the DSMB will be notified. As individuals with ASD often have cyclical changes in behavior independent of medication effects, the study staff will rely on caregivers' experience with the participant to determine if any observed behavior changes are within the expected range for that individual. If the investigational product is stopped permanently, the participant and his caregiver will be asked to complete the remainder of the study visits, return any unused study drug, and repeat safety lab studies.

2 Study Objectives

Primary Objective:

The primary objective of this clinical trial is to determine if Avmacol® is superior to placebo for improving social communication impairment in adolescent and young adult men with moderate to severe autism spectrum disorder (ASD), as measured by change in Social Responsiveness Scale-2 (SRS-2) [100] total scores.

Secondary Objectives:

Secondary objectives include: (1) assessing the safety and tolerability of Avmacol® in subjects with ASD; and (2) characterizing the effects of Avmacol® on behavioral challenges commonly associated with ASD, including hyperactivity, irritability, repetitive behavior, and inappropriate speech.

Exploratory Objectives:
In a subset of 30 participants, exploratory objectives include: (1) Assessment of gut micobiota diversity and relative abundance of microbial species from fecal samples collected before and after 12 weeks of treatment with sulforaphane or placebo. (2) Explore the relationship between changes in microbiota composition and behavioral outcomes in young men treated with sulforaphane or placebo.

3 Study Design

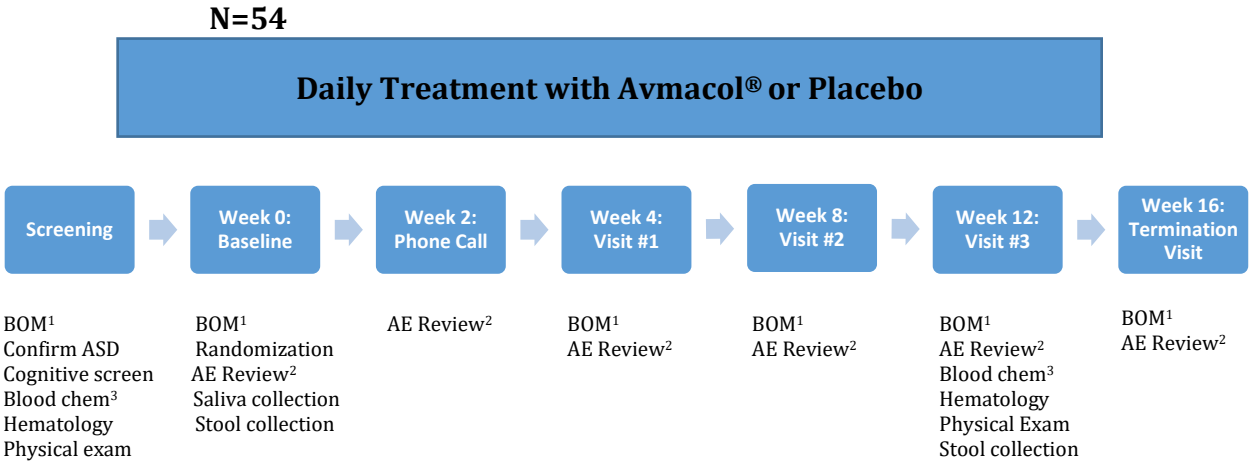
3.1 General Design

We propose to conduct a Phase II, randomized, double-blind, placebo-controlled trial of Avmacol®, a rich source of sulforaphane, for the treatment of social impairment in individuals with ASD. Fifty-four male subjects with autism spectrum disorder (ASD) between ages 13 and 30 years old will be randomized in a 1:1 ratio, stratified by age category (13-18 and 19-30 years old), to receive either Avmacol® or identical placebo tablets. Participation will be limited to males to closely approximate the sample used in the SF for ASD clinical trial by Singh et al. [48] and to account for possible mechanistic differences in ASD between males and females (ASD is 4 times more common in males). Stratification by age will account for possible age-based differences in response to treatment (adolescence vs. adulthood) and allow for secondary analysis by age group (adolescent vs. adult).

The length of study participation from baseline to study termination for each individual subject is 16 weeks, including a 12-week active treatment phase (Avmacol® or placebo), followed by a 4-week blinded discontinuation phase. Participants will be monitored with clinic visits at regular intervals (baseline and weeks 4, 8, 12, and 16) for improvement in a well-characterized, caregiver-rated measure of social impairment (Social Responsiveness Scale-2 (SRS-2) [100], the primary outcome) as well as secondary measures of (1) repetitive behaviors associated with ASD (Repetitive Behavior Scale- Revised; RBSR) [102]; (2) challenging behaviors commonly associated with developmental disabilities (Aberrant Behavior Checklist; ABC) [103]; (3) clinician-rated functional severity (Clinical Global Impression Scale- Severity; CGI-S) [104]; and clinician-rated functional improvement (Clinical Global Impression Scale- Improvement; CGI-I) [104].

Vital signs and reports of adverse events will be monitored at each study visit (Baseline, Week 4, Week 8, Week 12, Week 16) for tolerability and safety monitoring. A phone call with the subject's caregiver at Week 2 of the treatment phase will also be used to monitor for early adverse events. Physical exam and phlebotomy for routine laboratories (Complete Blood Count (CBC); Comprehensive Metabolic Panel (CMP); Liver Function Tests (LFTs), and Thyroid Stimulating Hormone (TSH)) will be conducted at Baseline and Week 12 for the purposes of safety monitoring. Fecal samples will be collected at home in the 48 hr prior to Baseline and Week 12 visits for microbiota analyses.

Schematic Diagram of Study Events:



[1] Behavioral outcome measures (SRS-2, ABC, CGI, RBSR)
[2] Adverse Events Review
[3] Blood chemistries (Comprehensive Metabolic Panel, Liver Function Tests, Thyroid Stimulating Hormone)

Primary Study Endpoints

The primary study endpoint will be percent change in mean SRS-2 total scores from Baseline to Week 12, the end of the active treatment phase.

Secondary Study Endpoints

- Percent change in mean SRS-2 total scores from Week 12 (the end of the active treatment phase) to Week 16 (the end of the discontinuation phase)
- Percent change in mean SRS-2 subscale scores (Social Awareness, Social Cognition, Social Communication, Social Motivation, and Restricted Interests/ Repetitive Behaviors) from Baseline to Week 12 and from Week 12 (the end of the active treatment phase) to Week 16 (the end of the discontinuation phase)
- Change in mean CGI-Severity score from Baseline to Week 12 and from Week 12 (the end of the active treatment phase) to Week 16 (the end of the discontinuation phase)
- Proportion of participants with CGI-Improvement (CGI-I) scores of 1 (very much improved) or 2 (much improved) in each treatment arm at Weeks 12 and Week 16
- Percent change in mean RBSR total scores from Baseline to Week 12 and from Week 12 (the end of the active treatment phase) to Week 16 (the end of the discontinuation phase).
- Percent change in mean ABC subscale scores (Social Withdrawal, Hyperactivity, Inappropriate Speech, Stereotypy, and Irritability) from Baseline to Week 12 and from Week 12 (the end of the active treatment phase) to Week 16 (the end of the discontinuation phase)
- Proportion of participants in each treatment arm meeting responder status, as defined by 10 point or greater reduction in SRS-2 total score AND CGI-I score of 1 (very much improved) or 2 (much improved).
- Proportion of participants in each group meeting partial responder status, as defined by 10 point or greater reduction in SRS-2 total score AND CGI-I score of 3 (minimally improved).

3.2 Primary Safety Endpoints

- CBC with differential, actual values and percent change in values from Screening visit to Week 12 visit.
- CMP (Na, K, Cl, CO2, BUN, Cr, Ca, Mg, Ph, glucose), actual values and percent change in values from Screening visit to Week 12 visit.
- LFTs (Total bilirubin, AST, ALT), actual values and percent change in values from Screening visit to Week 12 visit.
- TSH, actual value and percent change in value from Screening visit to Week 12 visit.
- Vital signs (weight, height, blood pressure, and heart rate), actual values at each time point measured and percent change in values from Baseline to Week 12 visit (the end of the active treatment phase)
- Physical Examination, general assessment of health at Screening visit and at Week 12 visit (the end of the active treatment phase)
- Mean (%) relative abundance of microbial phyla and genera in fecal samples at Baseline and Week 12.
- Percent change in Bacteroidetes to Firmicutes (B:F) ratio in fecal samples from Baseline to Week 12.
- Change in alpha diversity ("within-sample") metrics (the absolute number of operational taxonomic units (OTUs), Chao1 estimator, and Shannon index) in microbiota from Baseline to Week 12.
- Change in beta diversity ("between-sample") metrics (Bray-Curtis dissimilarity, Weighted Unifrac distance, and Unweighted Unifrac distance) in microbiota from Baseline to Week 12.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

- Males between ages 13-30 (inclusive) at the time of the consent
- Subject is capable of giving written informed consent or has a legally authorized representative (LAR) with sufficient intellectual capacity to provide written informed consent on the patient's behalf. For subjects that do not have sufficient capacity to provide informed consent, written informed assent will be obtained when developmentally appropriate.
- Subject has a reliable informant (parent or caregiver) who has sufficient past and current knowledge of the subject and will oversee the administration of study medication and accompany the subject to each study visit.
- Subject and caregiver have reliable means of transportation to attend study visits.
- Primary diagnosis of Autism Spectrum Disorder (ASD) severity level 2 or 3, confirmed by *Diagnostic and Statistical Manual-5* (DSM-5) criteria (as assessed by the PI, a physician experienced in the diagnosis of ASD) and meeting the autism cut-off score of 8 or greater on the Autism Diagnostic Observation Schedule-2 (ADOS-2) (conducted by a master's level

sub-investigator with extensive experience in administration of the ADOS-2) at the time screening

4.2 Exclusion Criteria

- Caregiver is unable to proficiently speak and understand English
- The subject is unable to ingest the study tablets orally, either by swallowing whole or crushing and mixing in a small amount of a soft food (e.g., applesauce, yogurt).
- Chronic medical illness that is not stable, would pose a risk to the participant if he participates in the trial, or would otherwise confound results (e.g. autoimmune disorders).
- A history of seizure within the 12 months preceding study enrollment
- Changes to psychopharmacological medications (e.g., stimulants, antidepressants, anxiolytics, antipsychotics) in the 2 weeks preceding study enrollment
- Significant changes to non-pharmacological treatments in the 4 weeks preceding study enrollment (e.g., starting a new behavioral therapy program)
- Chronic treatment with anti-inflammatory agents (e.g., ibuprofen, NSAIDs, corticosteroids)
- Clinically significant laboratory abnormalities at Screening visit (e.g., AST/ALT> two times the upper normal limits; serum creatinine > 1.2 mg/dl, TSH outside normal limits)
- Clinically significant findings on physical examination that investigator determines could increase risk of harm from participating in the study
- Participated in another clinical interventional trial or received an investigational product in the 30 days preceding study enrollment
- Previous therapeutic trial of sulforaphane or participation in a clinical trial in which sulforaphane was the investigational agent

4.3 Subject Recruitment and Screening

Subjects will primarily be recruited from the Carolina Institute for Developmental Disabilities (CIDD) Research Subject Registry, which includes more than 6000 families that have expressed an interest in research participation and have consented to be contacted about potential studies. Potential subjects will be contacted directly via email or letter by the Registry staff with information about the trial, including contact information of study staff. Participants will also be recruited from the clinical practices of physicians, psychologists, and allied health staff at the Carolina Institute for Developmental Disabilities as well as from local referring providers. Electronic and written advertisements will be disseminated to local community autism support agencies (e.g., Autism Society of North Carolina, Autism Resource Centers). All recruitment materials will first be approved by the University of North Carolina IRB.

Potential subjects expressing an interest in study participation will go through an initial telephone screening process to assess eligibility for study participation (e.g., previous autism diagnosis, male, ages 13-30, able to be accompanied by a caregiver who can serve as a primary informant, not taking chronic anti-inflammatory medication, free of major medical illness, no clinical seizures in the previous 12 months). Before the initial clinic screening visit, the caregiver and subject will be sent a copy of the informed consent form for review.

Written informed consent by a caregiver will be obtained before proceeding with all screening assessments. Whenever possible, informed assent will also be obtained from the participant. The study will pilot a tablet-based electronic decisional capacity tool developed at Research Triangle Institute (RTI) International to enhance participation in the assent process for individuals with developmental disabilities. Prior to study enrollment, participants will undergo diagnostic confirmation of autism spectrum disorder at the screening visit by review of DSM-5 Autism Spectrum Disorder (ASD) criteria (by the study PI) and by *Autism Diagnostic Observation Schedule-2* administration (by a master's level clinician experienced in ADOS-2 administration for research purposes). Screening requirements will also include routine laboratory testing (CBC, electrolytes, LFTs, TSH), medical history, and physical examination to identify any exclusionary factors. Screening laboratory values and physical exam findings must be within normal limits or deemed not clinically significant by the study physician prior to study enrollment. Subjects with serious medical illnesses that are poorly controlled, that may increase risks associated with study participation, or that may otherwise confound results will not be enrolled.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects will be withdrawn from *study* participation only if the subject or his legal authorized representative revokes consent for inclusion of subject data in group analyses.

Reasons for *treatment* withdrawal prior to the expected completion for a subject may include:

- The subject experiences a serious adverse event (SAE) that the study physician determines is likely related to the study treatment or that presents a safety risk for ongoing participation.
- The subject experiences a clinical seizure during the course of the active treatment phase, either by EEG confirmation or by strongly suggestive clinical history.
- The subject requires significant dose adjustments to concomitant psychopharmacological medications (e.g. stimulants, antidepressants, mood stabilizing agents, benzodiazepines) or starts a new psychopharmacological medication due to significantly worsened behavioral disturbance.
- The subject requires a new chronic medication that may potentially interact with the study medication.

No withdrawal symptoms or adverse effects are anticipated from abrupt discontinuation of the study medication. However, if a patient needs to discontinue the study treatment for one of the aforementioned reasons, every effort will be made to bring the subject in promptly for a clinic visit to perform safety measures, including physical examination and laboratory measures. As described below, study personnel will request that subjects who prematurely stop treatment complete the remaining study visits and procedures as outlined in the protocol.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

If a subject withdraws from treatment prematurely, every effort will be made to obtain the subject/ caregiver's permission to collect data through the end of the planned subject follow-up period (week 16 visit). The data collected will be included in the final data set under intention-to-treat analysis, provided the participant took at least 1 dose of study medication and attended at least 1 post-baseline assessment.

If a subject appears lost to follow-up, study personnel will attempt to reach the subject's caregiver by all provided telephone numbers and email addresses on two occasions each. If the caregiver cannot be reached by phone or email, a certified letter will be sent requesting that the caregiver contact the study personnel to further discuss the subject's involvement in the trial. A second certified letter will be sent two weeks after the first letter was sent if the subject's caregiver has not responded within those two weeks. If the caregiver does not respond to the second letter, the subject will be considered lost to follow-up. All attempts to contact the subject's caregiver will be documented in a communication log.

5 Study Drug

5.1 Description

The investigational agent is supplied as a white, flecked, uncoated tablet. Each active tablet will contain 125 mg broccoli seed extract powder (equivalent to approximately 36 µmol GR), 50 mg broccoli sprout extract powder (source of myrosinase), 15 mg ascorbic acid, 7.5 mg carboxymethylcellulose, 6.5 mg silicon dioxide, and 171 mg microcrystalline cellulose. The placebo tablets will be similar in shape, size, and color.

5.2 Treatment Regimen

Participants will take 3-8 Avmacol® or placebo tablets once daily by mouth for a total of 12 weeks, with dosing regimen based on weight as follows:

3 tablets (approx. 46.5 µmol SF) if <100 lb
5 tablets (approx. 77.5 µmol SF) if 100-125 lb
6 tablets (approx. 93 µmol SF) if 126-175 lb
7 tablets (approx. 108.5 µmol SF) if 176-199 lb
8 tablets (approx. 124 µmol SF) if ≥ 200 lb

The tablets may be crushed and mixed with a soft food, such as applesauce, if needed. Participants will be instructed to avoid taking the tablets with a heavy meal.

5.3 Method for Assigning Subjects to Treatment Groups

Enrolled participants will be stratified for age (13-18 and 19-30 years) to account for possible effects of age on treatment response. The goal is to recruit twenty-seven 13-18 year-olds and twenty-seven 19-30 year-olds to allow for separate analysis of adolescents and adults (exploratory). Individuals will be randomly assigned to treatment group within age strata by generating random numbers for each group for each entry (i.e., 1-27 for younger group and 1-27

for older group). The larger half of the random numbers within each group will be assigned to receive the treatment and the smaller half to the placebo condition. These two lists – listed in their original order (1-27 for younger and 1-27 for older) will be provided to UNC Investigational Drug Services (IDS) pharmacists. They will consult the list and provide the appropriate medication to each individual depending on the order in which they present to the pharmacy within their age group. Study personnel will remain blinded to treatment condition for the duration of the study.

5.4 Preparation and Administration of Study Drug

Avmacol® and placebo tablets will be shipped in bulk to UNC IDS (984-974-0469) in 500 count bottles, which will be stored at 2-8^{0}C in the refrigerator until needed. Prior to a participant's study visit, a 5-week supply of the investigational drug (Avmacol® or placebo) will be packaged in a smaller, labeled bottle to be picked up by study staff. The label will not indicate whether the bottles contain Avmacol® or placebo tablets. The study personnel will verify the number of tablets dispensed and record on the drug accountability form. The bottle of investigational drug will be stored in a small refrigerator used exclusively for this purpose at the study site until the designated visit. The investigational drug will be dispensed to the subject's caregiver at the time of the study visit (Baseline, Week 4, and Week 8 visits) in a freezer bag for transport with instructions to store the bottle in a home refrigerator. Subjects and caregivers will be advised to avoid taking the tablets with a heavy meal and to take by mouth at approximately the same time every day. If a subject is unable to swallow pills, the tablets may be crushed with a pill crusher or mortar and pestle and mixed with a soft food, such as applesauce. Of note, investigators at the Cullman Chemoprotection Center and others have successfully administered sulforaphane and its precursor, glucoraphanin, in a number of different formulations, both encapsulated and in various types of juices [43, 44, 46, 47, 105].

5.5 Subject Compliance Monitoring

Subjects' caregivers will be provided with a medication log to track daily dosing of the study medication. At Week 4, Week 8, and Week 12 visits, subjects and their caregivers will be instructed to return the medication log and any unused tablets to study staff, who will count the remaining tablets and address any discrepancies between remaining tablets and the medication log with the families. Compliance percentages will be recorded on a drug compliance form. For frequent missed doses, the study physician will discuss the importance of adherence to the recommended dosing regimen and will identify any potential barriers to compliance, providing support when possible.

5.6 Prior and Concomitant Therapy

Participants will be permitted to continue their usual regimen of medications, psychotropic and otherwise, during the study with the exception of chronic anti-inflammatory medications (e.g., ibuprofen, naproxen, corticosteroids), which may confound the treatment effects. Concomitant medication doses must be stable for 4 weeks prior to study enrollment and remain constant for the duration of the study, to the extent possible.

5.7 Packaging

Avmacol® will be shipped in bulk from Nutramax Laboratories, Inc. (2208 Lakeside Blvd, Edgewood, MD 21040) to UNC IDS in boxes of bottles containing 500 tablets of Avmacol© each. The bottle labels will include: product name (Avmacol®), manufacturer (Nutramax Laboratories, Inc.), Quantity, Lot Number, Expiration Date, Study Name (Avmacol® for autism spectrum disorder), Institution (UNC), PI (Politte), IRB number, "Investigational Study Material," and "Caution: New Drug Limited by Federal Law to Investigational Use Only." For an example of labelling, please see section 7.3.7 of the IND application.

Matched placebo tablets will be manufactured and shipped from the University of Maryland Department of Pharmaceutical Sciences (20 N. Pine St., Baltimore, MD 21201) to UNC IDS in boxes of HDPE plastic bottles containing 200 tablets each. The bottles will be individually labeled as described above.

5.8 Blinding of Study Drug (if applicable)

Subjects will be randomized by IDS pharmacy personnel using a random numbers list, and treatment assignment will not be disclosed to study staff. Avmacol® and placebo tablets will be similar in shape, size, and color, and the labels on bottles dispensed to study staff and families will not indicate treatment assignment. Blinding of treatment assignment for the study team and subjects will be maintained until after the conclusion of all data collection. In the event of a serious adverse event, a physician monitor who is not directly associated with the study (ie, an independent medical monitor) will determine if the SAE is potentially related to taking the study drug. If a possible association is suspected, the pharmacy will disclose treatment status to the medical monitor, and the event will be reviewed by the medical monitor and Data Safety Monitoring Board (DSMB) to determine if treatment discontinuation is warranted.

Receiving, Storage, Dispensing and Return

5.8.1 Receipt of Drug Supplies

Avmacol® will be shipped in bulk from Nutramax Laboratories, Inc. (2208 Lakeside Blvd, Edgewood, MD 21040) to UNC IDS (IDS Pharmacy, Room 3122N, UNC Hospitals, 101 Manning Drive, Chapel Hill, NC 27514) in boxes of bottles containing 500 tablets of Avmacol® each. Matched placebo tablets will be shipped in bulk from the University of Maryland Department of Pharamceutical Sciences (20 N. Pine St., Baltimore, MD 21201) to UNC IDS in boxes of bottles containing 200 placebo tablets each.

Upon receipt of the investigational drug/ placebo, an inventory will be performed and a drug receipt log filled out and signed by the IDS personnel accepting the shipment. IDS staff will count and verify that the shipment contains all of the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or placebo) will be documented in the study files. The PI will notify the manufacturer of any damaged or unusable study drug that was supplied to the study site.

5.8.2 Storage

The investigational drug (Avmacol®) and placebo tablets will be stored at 2-8^{0}C in the refrigerator in UNC Investigational Drug Services (IDS) pharmacy prior to being dispensed to study staff in the week prior to the designated study visit. The study drug will be stored on site at the CIDD in a refrigerator used expressly for study drug storage until the study visit, at which time it will be dispensed to the participant's caregiver by the study team. Caregivers will be asked to store the bottle of study drug in a home refrigerator as a precautionary measure to preserve the integrity of the compound, though the manufacturer of Avmacol® reports stability at room temperature.

5.8.3 Dispensing of Study Drug

Study drug will be dispensed by UNC IDS to study staff in labeled bottles containing a 5-week supply (4 week supply plus 1 week buffer) of active drug or placebo tablets prior to baseline, week 4, and week 8 study visits. The study drug will be stored on site at the CIDD in a refrigerator used expressly for study drug storage until the study visit, at which time it will be dispensed to the participant's caregiver by the study team. Caregivers will be asked to transport the investigational drug in a freezer bag and store the bottle of study drug in a home refrigerator as a precautionary measure to preserve the integrity of the compound, though the manufacturer of Avmacol® reports stability at room temperature. Caregivers will be asked to record each administered dose on a medication log and to return the medication log and any unused study drug to study staff at the next visit. Study drug reconciliation will be performed at week 4, week 8, and week 12 study visits to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug accountability form, and signed and dated by the study personnel and study physician.

Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to destruction of unused study drug by IDS. Drug destroyed on site will be documented in the study files.

6 Study Procedures

6.1 Pre-Screen Phone Call:

Prior to screening, potential participants will undergo a pre-screening assessment by telephone or direct clinical interview with study staff to determine if basic eligibility criteria are met. A written copy of the informed consent document will be provided by mail, electronically, or in person for the participant's caregiver to read prior to the screening visit.

6.2 Screening Visit:

The screening visit will take approximately 3 hours to complete. At screening, we will first obtain written informed consent from participants or their Legally Authorized Representatives (LAR). We anticipate that most subjects will not have capacity to provide informed consent themselves due to cognitive and/or communication deficits associated with ASD, though every effort will be

made to increase their participation in the consent/ assent process. For individuals under 18 years of age or those unable to consent due to significant cognitive or communication impairment, written assent for participation will be sought. An electronic, tablet-based interactive consent tool will be piloted to enhance participation in the consent process.

After written informed consent is obtained, diagnosis of autism spectrum disorder (ASD) will be confirmed by two measures: clinical review of the Diagnostic and Statistical Manual-5 (DSM-5) [1] ASD criteria and Autism Diagnostic Observation Schedule-2 (ADOS-2) total score [106]. The ADOS-2 is a semi-structured assessment of communication, social interaction, and repetitive behavior that is widely regarded as the gold standard of ASD diagnosis [106]. The ADOS-2 will be performed by a trained master's-level clinician, and DSM-5 diagnostic criteria for ASD will be assessed by a study physician with extensive experience in diagnosing ASD. ADOS-2 administration will be videotaped to permit clinician scoring after administration; the digital recording will be stored on a secure server than will be accessible only by authorized study personnel with a password. Individuals must meet criteria for a diagnosis of ASD based on both DSM-5 criteria (severity level 2 or 3) and ADOS-2 total score (≥8, exceeding the "autism" cut-off) to be eligible for participation. An abbreviated cognitive assessment using the Wechsler Abbreviated Intelligence Scales (WASI) two non-verbal subtests will be [107] obtained for the purposes of data analysis, though there is no cut-off intelligence quotient (IQ) score for study participation. A medical history will be obtained by the study physician, including current medical conditions, past medical history, surgical history, psychiatric history, past and current psychotropic medications, allergies, and current non-pharmacological treatments for ASD (e.g., speech therapy, occupational therapy, behavioral therapy). Caregivers will complete the Social Responsiveness Scale, Second Edition (SRS-2) [100], Repetitive Behavior Scale-Revised (RBSR) [102], and Aberrant Behavior Checklist- Community Edition (ABC) [103] for baseline characterization of social communication deficits, repetitive behaviors, and behavioral challenges. The study physician will complete the Clinical Global Impression Severity Scale (CGI-S) [104], a 7-point rating scale of overall symptom severity. Baseline bloodwork will include a comprehensive metabolic profile (CMP), liver function tests (LFTs), thyroid stimulating hormone (TSH), and complete blood count (CBC) with white blood count differential. Participants will be given an OMNIGene-Gut stabilization kit to take home and use in the collection of a stool sample in the 48 hr prior to the scheduled Baseline visit. The research team will instruct the participant and caregiver in the collection of the stool sample and ask them to return it at the Baseline visit.

6.3 Baseline Visit (Week 0):

The baseline visit will take approximately 1.5 hr to complete. Prior to the baseline visit, the study physician will review safety labs drawn at the screening visit (CBC, CMP, LFTs, TSH) to ensure eligibility for participation. For individuals who meet all inclusionary and exclusionary criteria as described in sections 4.1 and 4.2, a baseline visit will be scheduled within 2 weeks of screening. Clinical outcome measures will again be obtained from caregivers, including the SRS-2, ABC, and RBSR. The study physician will complete the CGI-S to establish baseline clinical severity of ASD prior to intervention. Vital signs (height, weight, blood pressure, heart rate, and temperature) will be obtained by study staff. An adverse event log for baseline symptom assessment will be completed by the study physician. With specific written informed consent, participants will provide a saliva sample (approximately 2.6 mL total), either through expectoration into a special collection tube or through cheek swab (approximately 5 swabs). The de-identified saliva sample

will be stored in the UNC Biospecimen Repository for use in a possible future study of genetic markers associated with clinical response to sulforaphane. Participation in the storage of genetic material is not required for participation in the primary clinical trial. Participants will also return the stool sample in the OMNIGene-Gut stabilization kit, which is stable at room temperature for up to 60 days. The kit will be labeled with the date and participant PIN, securely packaged in a Styrofoam container, and mailed to the Cullman Chemoprotection Center at JHU (director, Dr. Jed Fahey) for storage in cryovials at -80^{0}C until all study samples have been collected.

Participants will be randomly assigned at baseline to receive either the active compound (Avmacol®) or identical placebo tablets. UNC IDS will be provided with a random table of numbers for randomization of participants, and both study staff and participants will remain blinded to treatment assignment. Study staff will retrieve the investigational product in labeled bottles from IDS and stored on site in a refrigerator at 2-8^{0}C prior to the baseline visit. A 5-week supply of investigational drug will be dispensed to the participant and their caregiver in a labeled bottle by study staff at the visit, and they will be instructed to transport the bottle home in a freezer bag (supplied by study staff) and store the bottle in their home refrigerator to ensure compound stability (a precautionary measure; the manufacturer of Avmacol® reports stability at room temperature). They will be given detailed instructions for taking the medication and completing the medication log, which will be returned and reviewed at the participant's next visit. Caregivers will also be instructed to return any remaining tablets to the study staff at the following visit to determine adherence to the dosing protocol.

6.4 Week 2 Telephone Call:

The week 2 telephone call is expected to take approximately 20 minutes. The study physician will contact the subject's caregiver 2 weeks after the baseline visit to assess tolerability and general response. The adverse event log will be completed by the physician. Caregivers will be asked to report any changes in concurrent medications or non-pharmacological treatments since the previous study visit.

6.5 Week 4 Follow-Up Visit:

The week 4 visit will take approximately 1 hour. The subject and his caregiver will return to the clinic for a follow-up visit that will include measurement of vital signs (height, weight, blood pressure, heart rate, and temperature), caregiver completion of behavioral outcome measures (SRS-2, RBSR, and ABC), and clinician-rated CGI-Severity and CGI-Improvement scores. The adverse event log will also be completed by the study physician. Caregivers will be asked to report any changes in concurrent medications or non-pharmacological treatments since the previous study visit. The medication log and returned tablets will be reviewed/ counted for treatment compliance, and the next 5-week supply of investigational drug will be dispensed to the participant and his caregiver. Caregivers will be instructed to administer any unused tablets from the previous bottle before starting the new supply.

6.6 Week 8 Follow-Up Visit:

The week 8 visit will take approximately 1 hr. to complete. The patient and his caregiver will return to the clinic for a follow-up visit that will include measurement of vital signs (height, weight, blood pressure, heart rate, and temperature), caregiver completion of behavioral outcome measures (SRS-2, RBSR, and ABC), and clinician-rated CGI-Severity and CGI-Improvement scores.

The adverse event log will also be completed by the study physician. Caregivers will be asked to report any changes in concurrent medications or non-pharmacological treatments since the previous study visit. The medication log and returned tablets will be reviewed/ counted for treatment compliance, and the next 5-week supply of investigational drug will be dispensed to the participant and his caregiver. Caregivers will be instructed to administer any unused tablets from the previous bottle before starting the new supply. Participants will be given a new OMNIGene-Gut stabilization kit for collection of a stool sample at home in the 48 hr prior to the scheduled Week 12 visit. The research team will remind the participant and his caregiver of how to collect the sample and instruct to return it at the next visit.

6.7 Week 12 Follow-Up Visit:

The week 12 visit marks the end of the active treatment phase of the study and will take approximately 2 hr. to complete. The patient and his caregiver will return to the clinic for a follow-up visit that will include measurement of vital signs (height, weight, blood pressure, heart rate, and temperature), physical exam by the study physician, and repeat blood draw for safety monitoring (CMP, LFTs, TSH, and CBC with differential). Caregivers will complete behavioral outcome measures (SRS-2, RBSR, and ABC), and clinicians will assign CGI-Severity and CGI-Improvement scores. At this phase-ending visit, responder status will be assigned to those with a 10 point or greater reduction on the SRS-2 and CGI-Improvement score of 1 or 2 (very much or much improved). Partial responder status will be assigned to participants with a 10 point or greater reduction on the SRS-2 and a CGI-Improvement score of 3 (minimally improved). The adverse event log will also be completed by the study physician. Caregivers will be asked to report any changes in concurrent medications or non-pharmacological treatments since the previous study visit. The medication log and returned tablets will be reviewed/ counted for treatment compliance, and any remaining investigational drug will be collected by study staff and returned to IDS for disposal. Participants will also return a stool sample in the OMNIGene-Gut stabilization kit. The kit will be labeled with the date and participant PIN, securely packaged in a Styrofoam container, and mailed to the Cullman Chemoprotection Center at JHU (director, Dr. Jed Fahey) for storage in cryovials at -80^{0}C until all study samples have been collected.

6.8 Week 16 Follow-Up Visit/ Study Termination:

The week 16 visit will take approximately 1 hr. to complete. Participants and their caregivers will return for a follow-up visit 4 weeks after discontinuing the investigational treatment. The assessment will include measurement of vital signs (height, weight, blood pressure, heart rate, and temperature), caregiver completion of behavioral outcome measures (SRS-2, RBSR, and ABC), and clinician-rated CGI-Severity and CGI-Improvement scores. The adverse event log will also be completed by the study physician. Caregivers will be asked to report any changes in concurrent medications or non-pharmacological treatments since the previous study visit. Treatment assignment (Avmacol® or placebo) will be obtained by study staff from IDS and shared with participants and their caregiver. Participants who were assigned to take placebo will have the opportunity to receive a 3-month supply of Avmacol® at no cost, supervised by the study physician under routine clinical care following study exit. Monetary compensation for study participation will be provided to participants and their caregiver in the form of 2 checks at the end of each clinic visit, as described in section 12.3 below.

7 Statistical Plan

7.1 Sample Size Determination

We calculated power for comparing groups on change in SRS-2 total score (the primary outcome measure), using a standard approach for linear mixed models (Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O. *SAS for mixed models [electronic resource].* Cary, N.C.: SAS Institute, Inc.; 2006). We assumed that the mean differences observed in the trial by Singh et al. represented the true values (i.e., we assumed mean differences of 7, 20, 18.4, and 0.4 at weeks 4, 8, 12 and 16, respectively), and we assumed a constant standard deviation of 20, consistent with that observed by Singh et al. Furthermore, we assume within-participants across time correlation of 0.5 (a conservative value) and we assume no more than 15% loss distributed over the 16 weeks of follow-up. Under these assumptions, randomizing 27 participants to each group will provide at least 80% power for an overall test comparing groups across all time points using a two-sided 4 degree of freedom test at the 0.05 significance level.

For the exploratory microbiota analysis, we aim to include a subset of 30 subjects in the sample. In a recent randomized cross-over trial of the effects of broccoli consumption on microbiota composition in 18 healthy adults [Kaczmarek 2017*], participants consuming broccoli exhibited an increase in the ratio of Baceteroidetes to Firmicutes by 37% from baseline to end, compared to a 5% reduction during the control period (p=0.02). With 15 participants per group, we will have >80% power to detect a similar change in the ratio of Bacteroidetes to Firmicutes.

* Kaczmarek JL, et al. Broccoli consumption impacts the human gastrointestinal microbiota. The FASEB Journal, 2017. 31(1 suppl): p.965.

7.2 Statistical Methods

General linear mixed models (GLMM) will be used to test the primary and secondary hypotheses in longitudinal analyses:

Primary Hypotheses

Compared to control participants, treated participants will show larger changes over time on SRS-2 total scores from Baseline to Week 12, and differences will not be maintained after treatment discontinuation at Week 12 through the end of the follow-up period at Week 16.

GLMM analyses will describe individual patterns of change over time on the SRS-2 Total score. Individual intercepts and slopes will be estimated as random-effects variables, and will be allowed to correlate. The SRS-2 scores from Baseline, Week 4, 8, 12, and 16 will be modeled as a function of treatment, time, time x treatment and will include covariates of age-stratum and the SRS-2 total score from screening. In addition to time, we will add a time-varying variable representing treatment phase (0=treatment, 1=follow-up period) and we cross this variable with treatment, time, and treatment x time. Time will be centered at Week 12. Of interest will be the main effect of treatment (i.e., treatment impact at end of active treatment), treatment x time (i.e., treatment impact on change over time during the active treatment phase), and treatment x time x treatment phase (i.e., treatment differences in change over time during follow-up period). We will use an

unstructured covariance matrix. We will first test for any differences between groups across all four groups using a 4 degree of freedom linear contrast of the model parameters. If this overall test is significant at the 5% level, we will then test for differences separately at each time point. We will also report estimated mean differences along with 95% confidence intervals.

Secondary Hypotheses

Compared to control participants, treated participates will show larger changes over time on CGI Severity and RBSR scores from Baseline to Week 12 and will show reduction in those gains after discontinuation through the end of the follow-up period at Week 16.

Compared to control participants, treated participates will show larger changes over time on subscales when treatment impacts were observed on the total scores on the SRS-2, CGI Severity, and RBSR from Baseline to Week 12 and will show reduction in those gains after discontinuation through the end of the follow-up period at Week 16.

Similar GLMM analyses will be conducted to examine the longitudinal assessments of CGI Severity and RBSR and of subscales if the total scores show treatment impacts.

Exploratory Hypotheses

We hypothesize that treatment with sulforaphane will result in greater diversity of gut microbial species and a higher ratio of Bacteroidetes to Firmicutes spp. compared to placebo, and that these alterations in gut microbiota will be positively associated with behavioral improvements in the sulforaphane treatment group.

We will utilize Genotek's validated wet-lab and metagenomic data analytic pipeline for the batched sample processing and data analysis. Microbial DNA will be extracted and amplified using PCR, and 16S rRNA gene sequencing will be performed using the Illumina MiSeq platform. A curated taxonomic database will be used to assign a taxonomic classification to the sequencing reads. The mean (%) relative abundance of microbial phyla and genera will be calculated for the sulforaphane and placebo groups pre- and posttreatment. The percent change in Bacteroidetes to Firmicutes (B:F) ratio from baseline to endpoint will be compared between treatment groups using Analysis of Covariance (ANCOVA), with baseline B:F ratio included as a covariate.

Microbiota diversity and relative abundance will be assessed using conventional within sample ("alpha diversity") and between-sample ("beta diversity") metrics. Three metrics will be used to assess change in alpha diversity over time: the absolute number of operational taxonomic units (OTUs), Chao1 estimator, and Shannon index. We will use ANCOVA to test for significant differences in alpha diversity from baseline to endpoint within each treatment group. Beta diversity will be assessed with 3 metrics: Bray-Curtis dissimilarity, Weighted Unifrac distance, and Unweighted Unifrac distance. These indices will be used to determine the variance in an individual over time and the mean dissimilarity between individuals in each group. Weighted Unifrac distance will be used for Principal Coordinates Analysis (PCoA). We will create a PCoA plot to visually identify whether samples from each treatment group cluster together at different time points (before and after treatment). This can help determine whether any changes identified during beta diversity analysis are directed changes or random noise.

We will utilize our behavioral data collection to determine if changes in microbiota diversity and relative abundance are associated with behavioral improvement. Linear mixed models will be used to determine if microbiota composition changes over time covary with change in behavioral outcome scores.

7.3 Subject Population(s) for Analysis

Study analysis will include all available data from all randomized subjects who received at least one dose of study medication and participated in at least one post-baseline efficacy assessment (i.e., intent-to-treat population). Missing outcome data will not be imputed and will be assumed to be missing at random.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research,
- Serious (as defined below) "***Serious" is different than "severe" as reported in the CTC criteria that applies a grade to the AE.***

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries will be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Based on previous clinical trials of sulforaphane in humans [43, 48, 51], we do not anticipate any significant adverse events with Avmacol®, though the following gastrointestinal symptoms may potentially occur with greater frequency:

- Nausea and vomiting
- Abdominal pain
- Large or soft stools
- Constipation
- Diarrhea
- Weight gain

In a randomized, controlled trial of sulforaphane for autism spectrum disorder conducted by Singh et al. [48], 2 subjects with a history of seizures had single unprovoked seizures during the course of the study (one after 3 weeks on sulforaphane, one 3 weeks after discontinuing sulforaphane), and the possibility of seizure as a potential adverse effect of sulforaphane could not be ruled out. In our proposed study, a history of clinical seizure in the year prior to screening is an exclusionary criterion for safety reasons.

One subject enrolled in this trial experienced a worsening of behavioral symptoms that may or may not have been directly study related.

Adverse Event Reporting Period

The study period during which adverse events must be reported is defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality will be recorded as a preexisting condition. At interval study visits and at the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events will be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the study physician will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality will be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event. Any condition responsible for surgery will be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event log of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document, though grouped under one diagnosis.

All adverse events occurring during the study period will be recorded in the adverse event log. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that

are still ongoing at the end of the study period will be followed up, either by phone call or clinic visit, to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately to the FDA, UNC Biomedical IRB, and DSMB.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

The study PI will immediately report any serious adverse events to the FDA, UNC Biomedical IRB, and DSMB meeting the following 3 criteria:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.3.1 Investigator reporting: notifying the study sponsor

The proposed trial is investigator-initiated, and thus the investigator is also the sponsor. Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, will be reported to the FDA within 7 calendar days using FDA Form 3500A for mandatory reporting. SAEs meeting the criteria outlined in the beginning of section 8.3 will also be reporting to the UNC Biomedical IRB by the study PI via IRBIS (the IRB information system for UNC) within 7 calendar days of becoming aware of the adverse event. Significant new information on ongoing serious adverse events will be provided promptly to the FDA and UNC IRB. A written report of the SAE will also be provided to the DSMB for committee review within 14 calendar days of the event.

8.3.2 Investigator reporting:

For reportable deaths, the initial submission to the UNC IRB will be made by contacting the IRB Director or Associate Director. The AE/Unanticipated Problem Form is required as a follow up to the initial submission.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the UNC IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigator's brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects. If a large number of protocol deviations are identified, or if they are deemed to affect patient safety or the overall efficacy of the trial, the protocol deviations will also be reported to the DSMB.

8.3.3 Investigator reporting:

Not applicable, as this is a single-site study.

8.3.4 Sponsor reporting: Notifying the FDA

The study PI (study sponsor) will promptly complete a written IND safety report for any adverse event meeting the following criteria:

- ***Within 7 calendar days***
  Any study event that is:
  - associated with the use of the study drug
  - unexpected,
  - fatal or life-threatening, and

- ***Within 15 calendar days***

Any study event that is:

- associated with the use of the study drug,
- unexpected, and
- serious, but not fatal or life-threatening

-or-

- a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

Additional reporting requirements

The study PI (sponsor) will identify in IND safety reports all previous reports concerning similar adverse events and analyze the significance of the current event in light of the previous reports.

Reporting Process

Adverse events will be submitted on FDA Form 3500A or in a narrative format, along with FDA form 1571. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3.

8.4 Unblinding Procedures

The study team will make every effort to maintain blinding of treatment assignment for both subject and investigator for the entire duration of the individual subject's study participation. Treatment unblinding will be limited to the occurrence of a serious adverse event (SAE) that the independent medical monitor determines is possibly related to study treatment and study participation. In the event of such an unanticipated problem, study staff will contact UNC IDS via the on-call pager (919-216-9727) to report the occurrence of the event and request that treatment assignment status be disclosed to the independent medical monitor. The study staff and participant and caregiver will remain blinded to treatment. The treatment status will be faxed by IDS to the medical monitor and relevant medical personnel involved in treating the subject (e.g., emergency room physician, primary care physician). A narrative description of the SAE and treatment status will be documented in the CRF, and the FDA and UNC IRB will be promptly notified as specified above in Section 8.3.1.

8.5 Stopping Rules

- If a participant experiences significant adverse events that affect tolerability, may reasonably be related to taking the investigational product, and do not meet the protocol definition for a serious AE (including non-extreme behavioral changes), the dose may be reduced by approximately 30% according to the following dose modification schedule:

Weight category	Prescribed weight-based dose of glucoraphanin	Prescribed # of tablets	70% of weight-based dose (ie, 30% reduction)	Prescribed # tablets for reduced dose (rounded to nearest 15 mg increment)*
<100 lb	45 mg	3	31.5 mg	2
100-125 lb	75 mg	5	52.5 mg	3
126-175 lb	90 mg	6	63 mg	4
176-199 lb	105 mg	7	73.5 mg	5
≥ 200 lb	120 mg	8	84 mg	6

*Rounded down if equidistant between two 15 mg dosing increments

- If the dose is reduced during the course of the study, the participant will continue the lowered dose for the remainder of the study. If adverse events do not improve within 14 days of lowering the dose, the investigational product may be permanently discontinued at the discretion of the treating study physician, participant, and participant's guardian. If a participant exhibits extreme exacerbation of behavioral symptoms (such as self-injurious behavior or aggression) that do not improve substantially following a dose reduction and are determined "probably" or "definitely" related to taking the investigational product, the investigational product may be permanently discontinued sooner than 14 days post-reduction, and the DSMB will be notified. As individuals with ASD often have cyclical changes in behavior independent of medication effects, the study staff will rely on caregivers' experience with the participant to determine if any observed behavior changes are within the expected range for that individual. If the investigational product is stopped permanently, the participant and his caregiver will be asked to complete the remainder of the study visits, return any unused study drug, and repeat safety lab studies.
- If a participant has a seizure while taking the study medication, the study medication will be stopped, and the DSMB will be notified. The participant will be evaluated by the study PI and the participant's primary care physician, treated, and monitored appropriately for the duration of the study. The participant and his caregiver will be asked to return to complete the remainder of the study visits, return any unused study drug, and repeat safety lab studies.
- If the participant newly requires a long-term medication that is thought to interact adversely with the study medication, the study medication will be stopped. The participant and his caregiver will be asked to return to complete the remainder of the study visits, return any unused study drug, and repeat safety lab studies.
- If a participant experiences a serious adverse event that is thought to be related to taking the study medication (as determined by an independent medical monitor), the study medication will be permanently discontinued, and the appropriate overseeing regulatory organizations will be notified (FDA, IRB, and DSMB). The participant and his caregiver will

be asked to return to complete the remainder of the study visits, return any unused study drug, and repeat safety lab studies.

- Potential reasons for stopping the entire study include: occurrence of a grade 3(severe) or grade 4 (life-threatening) SAE in more than one participant that an independent medical monitor and DSMB believe is likely related to active treatment; new information from other clinical trials that indicate serious risks associated with oral administration of a sulforaphane product; the death of a participant that is determined to be related to the study intervention; and insufficient funds to complete study tasks.

8.6 Medical Monitoring

It is the primary responsibility of the Principle Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a data and safety-monitoring plan (DSMP) (see section 9 Auditing, Monitoring and Inspecting). A medical monitor (ie, a physician not directly affiliated with the study) will be retained to review any SAEs and determine if the SAE may reasonably be related to treatment status.

8.6.1 Internal Data and Safety Monitoring Board or DSMP

The North Carolina Translational and Clinical Science (NC TraCS) Institute Data and Safety Monitoring Board (DSMB) will provide additional safety and oversight for the proposed trial. The NC TraCS DSMB will consist of a chair, an ethicist, an epidemiologist, a biostatistician, and one or more clinical researchers, each serving three year terms (please see Attachment 6C, NC TraCS DSMB Charter, for a listing of current Committee members). The DSMB will review all SAEs and any seizure events, which the study PI will submit in writing to the DSMB chair within 7 calendar days of the event. The DSMB will also review safety information (abnormal baseline and end-of-treatment screening labs; unanticipated problems; significant physical exam findings) in a face-to-face meeting after every 10 participants have completed the study. The study PI will supply this information in a written summary to the DSMB prior to the meeting and will participate in the first part of the committee meeting to review the findings. The study PI will also complete the Annual Data Report form, provided by the UNC Biomedical IRB, and submit to the DSMB annually for review of all adverse event reports. All data presented to the DSMB will be de-identified to protect the confidentiality of participants. Due to the relatively small size and anticipation of minimal risk associated with this study, no interim data analysis is planned.

The DSMB staff or chair will prepare minutes of each meeting within ten (10) working days of each meeting. These minutes will be circulated and approved at the next regularly scheduled meeting of the DSMB. The DSMB will report their findings to the UNC Biomedical IRB, as appropriate, and will make any recommendations directly to the IRB and to the study PI.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject/ LAR authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

Source documents will be maintained behind two levels of locked doors (ie, in a locked room on a floor with badge-access only). Only study staff directly involved in data collection, entry, and analysis and independent monitoring personnel will have access to source documents.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.4 Records Retention

It is the investigator's responsibility to retain essential study documents for at least 3 years after the study is completed and the FDA is notified (in accordance with 21 CFR 312.62).

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan in Appendix 6B. The investigator will allocate adequate time for such monitoring activities.

Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to the UNC Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the investigator-sponsor before commencement of this study.

All subjects for this study and their caregivers will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject or Legally Authorized Representative (LAR), using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or LAR, and the investigator-designated research professional obtaining the consent. If the subject is unable to provide informed consent, every effort will be made to obtain informed assent for participation.

12 Study Finances

12.1 Funding Source

This study is financed through a collaborative grant from the University of North Carolina Nutrition Research Institute and with research funds from Dr. Politte's KL2 Career Development Award (NIH/NCATS KL2TR001109).

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study investigator-sponsor prior to participation in this study. All UNC investigators will follow the University conflict of interest policy.

12.3 Subject Stipends or Payments

Subjects' caregivers will be reimbursed $30 per study visit to cover travel expenses. Subjects will also be reimbursed $20 per study visit as a token of appreciation for their participation. Payment will be provided in the form of two separate checks (one to caregiver, one to subject) at the conclusion of the specified study visit.

13 Publication Plan

The sponsor-investigator will hold primary responsibility for publication of the results of the study.

14 References

1. Association, A.P., *Diagnostic and Statistical Manual of Mental Disorders 5th Ed.* 2013, Washington, DC: American Psychiatric Publishing.
2. *Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010.* MMWR Surveill Summ, 2014. **63**(2): p. 1-21.
3. Coury, D.L., et al., *Use of psychotropic medication in children and adolescents with autism spectrum disorders.* Pediatrics, 2012. **130 Suppl 2**: p. S69-76.
4. Politte, L.C., C.A. Henry, and C.J. McDougle, *Psychopharmacological interventions in autism spectrum disorder.* Harv Rev Psychiatry, 2014. **22**(2): p. 76-92.
5. Estes, M.L. and A.K. McAllister, *Immune mediators in the brain and peripheral tissues in autism spectrum disorder.* Nat Rev Neurosci, 2015. **16**(8): p. 469-86.
6. Napoli, E., et al., *Deficits in bioenergetics and impaired immune response in granulocytes from children with autism.* Pediatrics, 2014. **133**(5): p. e1405-10.
7. Frye, R.E. and S.J. James, *Metabolic pathology of autism in relation to redox metabolism.* Biomark Med, 2014. **8**(3): p. 321-30.
8. Napoli, E., S. Wong, and C. Giulivi, *Evidence of reactive oxygen species-mediated damage to mitochondrial DNA in children with typical autism.* Mol Autism, 2013. **4**(1): p. 2.
9. Rose, S., et al., *Intracellular and extracellular redox status and free radical generation in primary immune cells from children with autism.* Autism Res Treat, 2012. **2012**: p. 986519.
10. Gu, F., V. Chauhan, and A. Chauhan, *Impaired synthesis and antioxidant defense of glutathione in the cerebellum of autistic subjects: alterations in the activities and protein expression of glutathione-related enzymes.* Free Radic Biol Med, 2013. **65**: p. 488-96.
11. Rossignol, D.A. and R.E. Frye, *Evidence linking oxidative stress, mitochondrial dysfunction, and inflammation in the brain of individuals with autism.* Front Physiol, 2014. **5**: p. 150.
12. Chauhan, A., T. Audhya, and V. Chauhan, *Brain region-specific glutathione redox imbalance in autism.* Neurochem Res, 2012. **37**(8): p. 1681-9.
13. Giulivi, C., et al., *Mitochondrial dysfunction in autism.* JAMA, 2010. **304**(21): p. 2389-96.
14. Rose, S., et al., *Oxidative stress induces mitochondrial dysfunction in a subset of autistic lymphoblastoid cell lines.* Transl Psychiatry, 2014. **4**: p. e377.
15. Tang, G., et al., *Mitochondrial abnormalities in temporal lobe of autistic brain.* Neurobiol Dis, 2013. **54**: p. 349-61.

16. Li, X., et al., *Elevated immune response in the brain of autistic patients.* J Neuroimmunol, 2009. **207**(1-2): p. 111-6.
17. Chez, M.G., et al., *Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children.* Pediatr Neurol, 2007. **36**(6): p. 361-5.
18. Wei, H., et al., *IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation.* J Neuroinflammation, 2011. **8**: p. 52.
19. Xu, N., X. Li, and Y. Zhong, *Inflammatory cytokines: potential biomarkers of immunologic dysfunction in autism spectrum disorders.* Mediators Inflamm, 2015. **2015**: p. 531518.
20. Suzuki, K., et al., *Microglial activation in young adults with autism spectrum disorder.* JAMA Psychiatry, 2013. **70**(1): p. 49-58.
21. Vargas, D.L., et al., *Neuroglial activation and neuroinflammation in the brain of patients with autism.* Ann Neurol, 2005. **57**(1): p. 67-81.
22. Lucchina, L. and A.M. Depino, *Altered peripheral and central inflammatory responses in a mouse model of autism.* Autism Res, 2014. **7**(2): p. 273-89.
23. Morgan, J.T., et al., *Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism.* Biol Psychiatry, 2010. **68**(4): p. 368-76.
24. Ebert, D.H. and M.E. Greenberg, *Activity-dependent neuronal signalling and autism spectrum disorder.* Nature, 2013. **493**(7432): p. 327-37.
25. Curran, L.K., et al., *Behaviors associated with fever in children with autism spectrum disorders.* Pediatrics, 2007. **120**(6): p. e1386-92.
26. Frye, R.E. and D.A. Rossignol, *Mitochondrial dysfunction can connect the diverse medical symptoms associated with autism spectrum disorders.* Pediatr Res, 2011. **69**(5 Pt 2): p. 41r-7r.
27. Mehler, M.F. and D.P. Purpura, *Autism, fever, epigenetics and the locus coeruleus.* Brain Res Rev, 2009. **59**(2): p. 388-92.
28. Stetler, R.A., et al., *Heat shock proteins: cellular and molecular mechanisms in the central nervous system.* Prog Neurobiol, 2010. **92**(2): p. 184-211.
29. Chen, J., I. Alberts, and X. Li, *Dysregulation of the IGF-I/PI3K/AKT/mTOR signaling pathway in autism spectrum disorders.* Int J Dev Neurosci, 2014. **35**: p. 35-41.
30. Huber, K.M., et al., *Dysregulation of Mammalian Target of Rapamycin Signaling in Mouse Models of Autism.* J Neurosci, 2015. **35**(41): p. 13836-42.
31. Tang, G., et al., *Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits.* Neuron, 2014. **83**(5): p. 1131-43.
32. Kensler, T.W., et al., *Keap1-nrf2 signaling: a target for cancer prevention by sulforaphane.* Top Curr Chem, 2013. **329**: p. 163-77.
33. Kensler, T.W., N. Wakabayashi, and S. Biswal, *Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway.* Annu Rev Pharmacol Toxicol, 2007. **47**: p. 89-116.
34. Frye, R.E. and D.A. Rossignol, *Treatments for biomedical abnormalities associated with autism spectrum disorder.* Front Pediatr, 2014. **2**: p. 66.
35. Brandenburg, L.O., et al., *Sulforaphane suppresses LPS-induced inflammation in primary rat microglia.* Inflamm Res, 2010. **59**(6): p. 443-50.
36. Gan, L. and J.A. Johnson, *Oxidative damage and the Nrf2-ARE pathway in neurodegenerative diseases.* Biochim Biophys Acta, 2014. **1842**(8): p. 1208-18.
37. Schachtele, S.J., S. Hu, and J.R. Lokensgard, *Modulation of experimental herpes encephalitis-associated neurotoxicity through sulforaphane treatment.* PLoS One, 2012. **7**(4): p. e36216.
38. Wu, Q.Q., et al., *Sulforaphane protects H9c2 cardiomyocytes from angiotensin II-induced hypertrophy.* Herz, 2014. **39**(3): p. 390-6.
39. Wiczk, A., et al., *Sulforaphane, a cruciferous vegetable-derived isothiocyanate, inhibits protein synthesis in human prostate cancer cells.* Biochim Biophys Acta, 2012. **1823**(8): p. 1295-305.
40. Alumkal, J.J., et al., *A phase II study of sulforaphane-rich broccoli sprout extracts in men with recurrent prostate cancer.* Invest New Drugs, 2015. **33**(2): p. 480-9.
41. Kensler, T.W., et al., *Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, Qidong, People's Republic of China.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(11 Pt 1): p. 2605-13.
42. Bahadoran, Z., et al., *Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial.* Int J Food Sci Nutr, 2012. **63**(7): p. 767-71.

43. Egner, P.A., et al., *Rapid and sustainable detoxication of airborne pollutants by broccoli sprout beverage: results of a randomized clinical trial in China.* Cancer Prev Res (Phila), 2014. **7**(8): p. 813-23.
44. Kensler, T.W., et al., *Modulation of the metabolism of airborne pollutants by glucoraphanin-rich and sulforaphane-rich broccoli sprout beverages in Qidong, China.* Carcinogenesis, 2012. **33**(1): p. 101-7.
45. Clarke, J.D., et al., *Metabolism and tissue distribution of sulforaphane in Nrf2 knockout and wild-type mice.* Pharm Res, 2011. **28**(12): p. 3171-9.
46. Egner, P.A., et al., *Bioavailability of Sulforaphane from two broccoli sprout beverages: results of a short-term, cross-over clinical trial in Qidong, China.* Cancer Prev Res (Phila), 2011. **4**(3): p. 384-95.
47. Fahey, J.W., et al., *Sulforaphane Bioavailability from Glucoraphanin-Rich Broccoli: Control by Active Endogenous Myrosinase.* PLoS One, 2015. **10**(11): p. e0140963.
48. Singh, K., et al., *Sulforaphane treatment of autism spectrum disorder (ASD).* Proc Natl Acad Sci U S A, 2014. **111**(43): p. 15550-5.
49. Fahey, J.W., A.T. Zalcmann, and P. Talalay, *The chemical diversity and distribution of glucosinolates and isothiocyanates among plants.* Phytochemistry, 2001. **56**(1): p. 5-51.
50. Fahey, J.W., Y. Zhang, and P. Talalay, *Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens.* Proc Natl Acad Sci U S A, 1997. **94**(19): p. 10367-72.
51. Shapiro, T.A., et al., *Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study.* Nutr Cancer, 2006. **55**(1): p. 53-62.
52. Shapiro, T.A., et al., *Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in humans.* Cancer Epidemiol Biomarkers Prev, 2001. **10**(5): p. 501-8.
53. Zhang, Y., *Role of glutathione in the accumulation of anticarcinogenic isothiocyanates and their glutathione conjugates by murine hepatoma cells.* Carcinogenesis, 2000. **21**(6): p. 1175-82.
54. Zhang, Y. and E.C. Callaway, *High cellular accumulation of sulphoraphane, a dietary anticarcinogen, is followed by rapid transporter-mediated export as a glutathione conjugate.* Biochem J, 2002. **364**(Pt 1): p. 301-7.
55. Brooks, J.D., V.G. Paton, and G. Vidanes, *Potent induction of phase 2 enzymes in human prostate cells by sulforaphane.* Cancer Epidemiol Biomarkers Prev, 2001. **10**(9): p. 949-54.
56. Fahey, J.W. and P. Talalay, *Antioxidant functions of sulforaphane: a potent inducer of Phase II detoxication enzymes.* Food Chem Toxicol, 1999. **37**(9-10): p. 973-9.
57. Ye, L., et al., *Quantitative determination of dithiocarbamates in human plasma, serum, erythrocytes and urine: pharmacokinetics of broccoli sprout isothiocyanates in humans.* Clin Chim Acta, 2002. **316**(1-2): p. 43-53.
58. Zhao, J., et al., *Sulforaphane enhances aquaporin-4 expression and decreases cerebral edema following traumatic brain injury.* J Neurosci Res, 2005. **82**(4): p. 499-506.
59. Jones, S.B. and J.D. Brooks, *Modest induction of phase 2 enzyme activity in the F-344 rat prostate.* BMC Cancer, 2006. **6**: p. 62.
60. Myzak, M.C., et al., *Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects.* Exp Biol Med (Maywood), 2007. **232**(2): p. 227-34.
61. Dinkova-Kostova, A.T., et al., *Protection against UV-light-induced skin carcinogenesis in SKH-1 high-risk mice by sulforaphane-containing broccoli sprout extracts.* Cancer Lett, 2006. **240**(2): p. 243-52.
62. Zhang, Y., et al., *Induction of GST and NQO1 in cultured bladder cells and in the urinary bladders of rats by an extract of broccoli (Brassica oleracea italica) sprouts.* J Agric Food Chem, 2006. **54**(25): p. 9370-6.
63. Barcelo, S., et al., *CYP2E1-mediated mechanism of anti-genotoxicity of the broccoli constituent sulforaphane.* Carcinogenesis, 1996. **17**(2): p. 277-82.
64. Rose, S., et al., *Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain.* Transl Psychiatry, 2012. **2**: p. e134.
65. Mead, J. and P. Ashwood, *Evidence supporting an altered immune response in ASD.* Immunol Lett, 2015. **163**(1): p. 49-55.
66. James, S.J., et al., *Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism.* Am J Med Genet B Neuropsychiatr Genet, 2006. **141b**(8): p. 947-56.
67. James, S.J., et al., *Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism.* Faseb j, 2009. **23**(8): p. 2374-83.
68. Dinkova-Kostova, A.T. and P. Talalay, *Direct and indirect antioxidant properties of inducers of cytoprotective proteins.* Mol Nutr Food Res, 2008. **52 Suppl 1**: p. S128-38.

69. Han, J.M., et al., *Protective effect of sulforaphane against dopaminergic cell death.* J Pharmacol Exp Ther, 2007. **321**(1): p. 249-56.
70. Kraft, A.D., D.A. Johnson, and J.A. Johnson, *Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult.* J Neurosci, 2004. **24**(5): p. 1101-12.
71. Mas, S., et al., *Sulforaphane protects SK-N-SH cells against antipsychotic-induced oxidative stress.* Fundam Clin Pharmacol, 2012. **26**(6): p. 712-21.
72. Mizuno, K., et al., *Glutathione biosynthesis via activation of the nuclear factor E2-related factor 2 (Nrf2)--antioxidant-response element (ARE) pathway is essential for neuroprotective effects of sulforaphane and 6-(methylsulfinyl) hexyl isothiocyanate.* J Pharmacol Sci, 2011. **115**(3): p. 320-8.
73. Tarozzi, A., et al., *Sulforaphane as a potential protective phytochemical against neurodegenerative diseases.* Oxid Med Cell Longev, 2013. **2013**: p. 415078.
74. Benedict, A.L., et al., *Neuroprotective effects of sulforaphane after contusive spinal cord injury.* J Neurotrauma, 2012. **29**(16): p. 2576-86.
75. Morroni, F., et al., *Neuroprotective effect of sulforaphane in 6-hydroxydopamine-lesioned mouse model of Parkinson's disease.* Neurotoxicology, 2013. **36**: p. 63-71.
76. Goh, S., et al., *Mitochondrial dysfunction as a neurobiological subtype of autism spectrum disorder: evidence from brain imaging.* JAMA Psychiatry, 2014. **71**(6): p. 665-71.
77. Chen, S., et al., *Elevated mitochondrial DNA copy number in peripheral blood cells is associated with childhood autism.* BMC Psychiatry, 2015. **15**: p. 50.
78. Rossignol, D.A. and R.E. Frye, *Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis.* Mol Psychiatry, 2012. **17**(3): p. 290-314.
79. Frye, R.E., et al., *Redox metabolism abnormalities in autistic children associated with mitochondrial disease.* Transl Psychiatry, 2013. **3**: p. e273.
80. Liu, H., P. Talalay, and J.W. Fahey, *Biomarker-Guided Strategy for Treatment of Autism Spectrum Disorder (ASD).* CNS Neurol Disord Drug Targets, 2016. **15**(5): p. 602-13.
81. Frye, R.E., S. Melnyk, and D.F. Macfabe, *Unique acyl-carnitine profiles are potential biomarkers for acquired mitochondrial disease in autism spectrum disorder.* Transl Psychiatry, 2013. **3**: p. e220.
82. Filipek, P.A., et al., *Relative carnitine deficiency in autism.* J Autism Dev Disord, 2004. **34**(6): p. 615-23.
83. Armah, C.N., et al., *A diet rich in high-glucoraphanin broccoli interacts with genotype to reduce discordance in plasma metabolite profiles by modulating mitochondrial function.* Am J Clin Nutr, 2013. **98**(3): p. 712-22.
84. Carrasco-Pozo, C., K.N. Tan, and K. Borges, *Sulforaphane is anticonvulsant and improves mitochondrial function.* J Neurochem, 2015. **135**(5): p. 932-42.
85. Brose, R.D., et al., *Activation of the stress proteome as a mechanism for small molecule therapeutics.* Hum Mol Genet, 2012. **21**(19): p. 4237-52.
86. Takano, T., *Role of Microglia in Autism: Recent Advances.* Dev Neurosci, 2015. **37**(3): p. 195-202.
87. Innamorato, N.G., et al., *The transcription factor Nrf2 is a therapeutic target against brain inflammation.* J Immunol, 2008. **181**(1): p. 680-9.
88. Horowitz, M. and S.D. Robinson, *Heat shock proteins and the heat shock response during hyperthermia and its modulation by altered physiological conditions.* Prog Brain Res, 2007. **162**: p. 433-46.
89. Wiggins, J.L., et al., *Using a self-organizing map algorithm to detect age-related changes in functional connectivity during rest in autism spectrum disorders.* Brain Res, 2011. **1380**: p. 187-97.
90. Gan, N., et al., *Sulforaphane activates heat shock response and enhances proteasome activity through up-regulation of Hsp27.* J Biol Chem, 2010. **285**(46): p. 35528-36.
91. Prestera, T., et al., *Parallel induction of heme oxygenase-1 and chemoprotective phase 2 enzymes by electrophiles and antioxidants: regulation by upstream antioxidant-responsive elements (ARE).* Mol Med, 1995. **1**(7): p. 827-37.
92. Sawicka, K. and R.S. Zukin, *Dysregulation of mTOR signaling in neuropsychiatric disorders: therapeutic implications.* Neuropsychopharmacology, 2012. **37**(1): p. 305-6.
93. Shehata, M. and K. Inokuchi, *Does autophagy work in synaptic plasticity and memory?* Rev Neurosci, 2014. **25**(4): p. 543-57.
94. Pawlik, A., et al., *Sulforaphane inhibits growth of phenotypically different breast cancer cells.* Eur J Nutr, 2013. **52**(8): p. 1949-58.
95. Jo, C., et al., *Sulforaphane induces autophagy through ERK activation in neuronal cells.* FEBS Lett, 2014. **588**(17): p. 3081-8.

96. Liu, Y., et al., *Sulforaphane enhances proteasomal and autophagic activities in mice and is a potential therapeutic reagent for Huntington's disease.* J Neurochem, 2014. **129**(3): p. 539-47.
97. Suppipat, K., et al., *Sulforaphane induces cell cycle arrest and apoptosis in acute lymphoblastic leukemia cells.* PLoS One, 2012. **7**(12): p. e51251.
98. Riedl, M.A., A. Saxon, and D. Diaz-Sanchez, *Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway.* Clin Immunol, 2009. **130**(3): p. 244-51.
99. Cipolla, B.G., et al., *Effect of Sulforaphane in Men with Biochemical Recurrence after Radical Prostatectomy.* Cancer Prev Res (Phila), 2015.
100. Constantino, J.N., et al., *Validation of a brief quantitative measure of autistic traits: comparison of the social responsiveness scale with the autism diagnostic interview-revised.* J Autism Dev Disord, 2003. **33**(4): p. 427-33.
101. McCracken, J.T., et al., *Risperidone in children with autism and serious behavioral problems.* N Engl J Med, 2002. **347**(5): p. 314-21.
102. Lam, K.S. and M.G. Aman, *The Repetitive Behavior Scale-Revised: independent validation in individuals with autism spectrum disorders.* J Autism Dev Disord, 2007. **37**(5): p. 855-66.
103. Aman, M.G., et al., *The aberrant behavior checklist: a behavior rating scale for the assessment of treatment effects.* Am J Ment Defic, 1985. **89**(5): p. 485-91.
104. Roid, G.H. (2003) Stanford-Binet Intelligence Scales, 5th ed. Itasca, IL: Riverside Publishing.
105. Fahey, J.W., et al., *Protection of humans by plant glucosinolates: efficiency of conversion of glucosinolates to isothiocyanates by the gastrointestinal microflora.* Cancer Prev Res (Phila), 2012. **5**(4): p. 603-11.
106. Lord, C., et al., *Autism diagnostic observation schedule: a standardized observation of communicative and social behavior.* J Autism Dev Disord, 1989. **19**(2): p. 185-212.
107. Wechsler, D., *Wechsler Abbreviated Scale of Intelligence*. 1999, The Psychological Corporation: San Antonio, TX.

15 Appendix 6A: Schedule of Study Events

Visit	*Consent, Eligibility (ADOS-2, DSMI-5 criteria)*	Medical history	Physical Exam	WASI	Vital signs	AE Review	Behavioral outcome measures (SRS-2; ABC; RBSR; CGI-S,I)	Safety labs (CBC, Chem, LFTs, TSH)	Saliva collection for storage	Stool collection for microbiota analysis	Visit Duration
Screen	**X**	**X**	**X**	**X**	**X**		**X**	**X**			**3 hr**
Week 0 Baseline					**X**	**X**	**X**		**X**	**X**	**1.5 hr**
Week 2 Phone call						**X**					**20 min**
Week 4					**X**	**X**	**X**				**1 hr**
Week 8					**X**	**X**	**X**				**1 hr**
Week 12 Treatment End			**X**		**X**	**X**	**X**	**X**		**X**	**2 hr**
Week 16 Study End					**X**	**X**	**X**				**1 hr**

16 Appendix 6B: Data and Safety Monitoring Plan

I. RISKS TO SUBJECTS

a. Human Subjects Involvement and Characteristics: Approval from the UNC IRB for this research project will be obtained prior to any recruitment or involvement of human subjects. The study will include 54 healthy adolescent and young adult males, ages 13-30 years old, with a diagnosis of Autism Spectrum Disorder (ASD) severity level 2 or 3, confirmed by review of *DSM-5* diagnostic criteria for ASD, and exceeding the ASD cut-off score of 8 on the Autism Diagnostic Observation Scheduled-2 (ADOS-2). The study population will be limited to male subjects for two primary reasons: (a) we aim to conduct an independent replication of a previous clinical trial of sulforaphane in ASD [1], which included only male participants; (b) potential mechanistic differences in ASD between males and females may lead to excessive heterogeneity in this relatively small sample size (ASD is 4 times more common in males than females). The proposed study includes adolescents and adults with developmental disabilities, both vulnerable populations, rather than healthy volunteers because the study intervention, a nutraceutical containing broccoli extracts, may provide specific benefit for social deficits observed in ASD, but is not expected to confer behavioral benefits for those without ASD. The intervention also has a well-documented safety profile and is considered low-risk. Furthermore, interventions in children with developmental disorders are likely to have greater impact than similar treatments at a later stage of development due to increased neuroplasticity early in development. The study will exclude individuals with significant medical problems and active seizure disorders (defined as clinical seizure activity in the 12 months preceding the study) due to theoretical increased risk of seizure with sulforaphane treatment [1]. Individuals taking chronic anti-inflammatory agents, such as corticosteroids or non-steroidal anti-inflammatory drugs, will also be excluded due to potential confounding of results.

Subjects will primarily be recruited from the Carolina Institute for Developmental Disabilities Research Subject Registry, which includes more than 6000 families that have expressed an interest in research participation and have consented to be contacted about potential studies. Potential subjects will be contacted directly via email or letter by the Registry staff with information about the trial, including contact information of study staff. Participants will also be recruited from the clinical practices of physicians, psychologists, and allied health staff at the Carolina Institute for Developmental Disabilities as well as from referring providers. Electronic and written advertisements will be disseminated to local community autism support agencies (e.g., Autism Society of North Carolina, Autism Resource Centers). All advertisement materials will first be approved by the University of North Carolina IRB.

Enrolled participants will be stratified for age (13-18 and 19-30 years) to account for possible effects of age on treatment response. The goal is to recruit twenty-seven 13-18 year-olds and twenty-seven 19-30 year-olds to allow for secondary analysis by age group. Individuals will be randomly assigned to treatment group within age strata by generating random numbers for each group for each entry (i.e., 1-27 for younger group and 1-27 for older group). The larger half of the random numbers within each group will be assigned to receive the treatment and the smaller half to the control condition. These two lists – listed in their original order (1-27 for younger and 1-27 for older) will be provided to UNC Investigational Drug Services (IDS) pharmacists. They will consult the list and provide the appropriate medication to each individual depending on the order in which they present to the pharmacy within their age group. Study personnel will remain blinded to treatment condition for the duration of the study.

During the course of the study, participants will take 3-8 Avmacol© or placebo tablets once daily by mouth for a total of 12 weeks, with dosing regimen based on weight as follows:

3 tablets (approx. 46.5 µmol SF) daily if <100 lb
5 tablets (approx. 77.5 µmol SF) daily if 100-125 lb
6 tablets (approx. 93 µmol SF) daily if 126-175 lb
7 tablets (approx. 108.5 µmol SF) daily if 176-199 lb
8 tablets (approx. 124 µmol SF) daily if ≥ 200 lb

If unable to swallow the requisite number of tablets whole, subjects will have the option to crush the tablets and mix with a soft food, such as applesauce or yogurt, for administration.

If a participant experiences significant adverse events that affect tolerability, may reasonably be related to taking the investigational product, and do not meet the protocol definition for a serious AE (including behavioral changes that are not severe), the dose may be reduced by approximately 30% according to the following dose modification schedule:

Weight category	Prescribed weight-based dose of glucoraphanin	Prescribed # of tablets	70% of weight-based dose (ie, 30% reduction)	Prescribed # tablets for reduced dose (rounded to nearest 15 mg increment)*
<100 lb	45 mg	3	31.5 mg	2
100-125 lb	75 mg	5	52.5 mg	3
126-175 lb	90 mg	6	63 mg	4
176-199 lb	105 mg	7	73.5 mg	5
≥ 200 lb	120 mg	8	84 mg	6

*Rounded down if equidistant between two 15 mg dosing increments

If the dose is reduced during the course of the study, the participant will continue the lowered dose for the remainder of the study. If adverse events do not improve within 14 days of lowering the dose, the investigational product may be permanently discontinued at the discretion of the treating study physician, participant, and participant's guardian. If a participant exhibits extreme exacerbation of behavioral symptoms (such as self-injurious behavior or aggression) that do not improve substantially following a dose reduction and are determined "probably" or "definitely" related to taking the investigational product, the investigational product may be permanently discontinued sooner than 14 days post-reduction, and the DSMB will be notified. As individuals with ASD often have cyclical changes in behavior independent of medication effects, the study staff will rely on caregivers' experience with the participant to determine if any observed behavior changes are within the expected range for that individual. If the investigational product is stopped permanently, the participant and his caregiver will be asked to complete the remainder of the study visits, return any unused study drug, and repeat safety lab studies.

b. Source of Materials: Data collection will primarily include clinical and behavioral information, including: medical history, medication/treatment history, developmental history, behavioral questionnaires/ inventories, clinical assessment for ASD, cognitive evaluation, report of adverse events, and physical examination. Phlebotomy will be performed at screening and at the end of the active treatment phase (Week 12 visit) for (a) basic safety lab monitoring (complete blood count, comprehensive metabolic panel, liver function tests, and thyroid stimulating hormone). Stool samples will be collection at Baseline and Week 12 for analysis of microbiota diversity and relative abundance.

c. Potential Risks: Cruciferous plants, including broccoli sprouts, are widely regarded as safe for ingestion and are regular dietary components in many regions of the world. Based on previous data from clinical trials of broccoli sprout extracts (BSE), we anticipate few, if any, adverse effects, as summarized below:

A Phase I study of orally administered sulforaphane- and glucoraphanin-containing BSE (75 µmol glucosinolates, 300 µmol glucosinolates, or 75 µmol isothiocyanates daily) for 7 days in 12 healthy volunteers did not demonstrate any clinical adverse events [2]. Two of 12 subjects assigned to active treatment demonstrated an increase in plasma ALT exceeding the upper limit of normal, though ALT levels rose for all subjects during the course of the study (including those assigned to placebo control), and the increase was not clearly attributable to consumption of BSE. Three of 12 subjects (2 active treatment, 1 placebo) demonstrated TSH levels exceeding the upper limit of normal during or after the dosing period, though elevated TSH was not associated with clinical symptoms or T3/T4 abnormalities.

Egner et al. [3] conducted a cross-over trial in 50 participants, randomized to ingest a sulforaphane-rich beverage (150 µmol/day) for 7 days in a first or second phase. Two of 50 participants complained of bitter taste or nausea, and no other adverse events were reported. No abnormal serum chemistry values were reported after 7 days. In a study of healthy participants ingesting a beverage containing 600 µmol of glucoraphanin and 40 µmol of sulforaphane, the intervention was well-tolerated with no laboratory evidence of effect on liver and kidney function [4].

A study of orally administered broccoli sprout homogenates (25 to 300 g) in 57 subjects over 3 separate days found no adverse effects aside from mild digestive effects [5]. Similarly, in a RCT of 20 men with recurrent prostate cancer treated with sulforaphane 200 µmol/ day for 20 weeks, no serious adverse events occurred, though some reported mild gastrointestinal upset [6].

A randomized, placebo-controlled trial of the sulforaphane product Prostaphane® in 81 men with rising PSA values following radical prostatectomy for prostate cancer represents the highest daily dosing of sulforaphane to date (338 µmol) [7]. No serious adverse events were reported, though gastrointestinal bloating was experienced somewhat more often in the SF group (N=17 vs. N=10), and one participant in the SF group withdrew from the study due to bowel discomfort. The differences in symptoms between the two groups were not statistically significant (p=0.149). Furthermore, no significant between-group differences were found for body weight, BMI, blood counts, and chemistries.

In a RCT of 45 male subjects with autism, ages 13-30, administered sulforaphane 50-150 µmol/day for 16 weeks, no significant differences in adverse events or safety labs (blood counts, chemistries, liver function tests, TSH) were reported [1]. Two subjects with a history of seizures had single unprovoked seizures during the course of the study (one after 3 weeks on sulforaphane, one 3 weeks after

discontinuing sulforaphane), and the possibility of seizure as a potential adverse effect of sulforaphane could not be ruled out.

One subject enrolled in the current trial experienced a worsening of behavioral symptoms that may or may not have been directly study related.

Finally, there is some evidence from animal studies and in vitro studies suggesting that sulforaphane and/or its metabolites can inhibit expression of CYP 1A2, 3A4, and 2D6, which could be a potential source of drug-drug interactions if the participant is taking medications that are substrates of these enzymes. The risk of possible drug-drug interactions is not well-established. Upon reviewing the participants concurrent medications at screening and all subsequent visits, the study physician will consult an extensive P450 Table to identify possible drug-drug interactions. If the participant develops significant adverse effects that appear consistent with increased exposure to a concurrent medication metabolized by 1A2, 3A4, or 2D6, the dose of SF will be lowered by 30% as specified in the protocol. If the adverse events persist 14-day post-reduction, SF may be permanently discontinued at the discretion of the study physician, participant, and guardian.

In summary, based on available data from human clinical trials of SF-rich compounds, SF appears to be extremely well-tolerated with minimal risk aside from mild gastrointestinal symptoms, which may include bloating, nausea, and loose stools. No significant changes in blood counts, serum chemistries, liver function, kidney function, or thyroid function are anticipated. As it is unclear if sulforaphane may lower the seizure threshold in those with a history of seizures, our study will exclude those with a history of seizure within the previous year. As with any medication, there is a theoretical risk of allergic reaction, and participants and their caregivers will be instructed on how to identify possible signs of allergic reaction. Additional risks include mild physical discomfort, psychological distress, or mild bruising and bleeding associated with phlebotomy. Participants will be offered the use of a local anesthetic (EMLA cream) prior to blood draw to minimize physical discomfort, and there is a small risk of transient local reaction to EMLA cream, including paleness, erythema, and edema. Many individuals with ASD experience oral sensory aversions, and some could find taking multiple medication tablets on a daily basis unpleasant.

Though every effort will be taken to ensure the protection of confidential information, there is a small risk to privacy in the event that security measures are breeched. In addition, participants may experience some mild anxiety or psychological distress in answering questions about their psychiatric and medical history.

II. ADEQUACY OF PROTECTION AGAINST RISKS

a. **Recruitment and Informed Consent:** Subjects will primarily be recruited from the Carolina Institute for Developmental Disabilities Research Subject Registry, which includes more than 6000 families that have expressed an interest in research participation and have consented to be contacted about potential studies. Potential subjects will be contacted directly via email or letter by the Registry staff with information about the trial, including contact information of study staff. Participants will also be recruited from the clinical practices of physicians, psychologists, and allied health staff at the Carolina Institute for Developmental Disabilities as well as from referring providers. Electronic and written advertisements will be disseminated to local community autism support agencies (e.g., Autism

Society of North Carolina, Autism Resource Centers). All advertisement materials will first be approved by the University of North Carolina IRB.

Participants and their caregivers/ Legally Authorized Representatives (LARs) will be provided with a written copy of the Informed Consent document to read thoroughly prior to the initial screening visit. Due to social communication and cognitive deficits inherent in ASD, we anticipate that most participants, including those 18 years and older, will not be capable of providing adequate informed consent. For subjects unable to provide informed consent, either due to age or cognitive disability, every effort will be made to obtain written informed assent. To enhance the ability of participants to participate meaningfully in the consent/ assent process, a novel electronic tablet-based consent tool will be utilized during the consent process. Informed consent from the caregiver or participant will be obtained prior to conducting any study-related measures or activities. Participants or their LARs will sign a separate stored specimen consent form for the de-identified storage of a saliva specimen for possible future genetic analysis. Subjects will still be allowed to participate in the primary clinical trial if they do not consent for specimen storage.

b. Protection against risk: Risks to participants will be minimized through frequent follow-up and careful consideration of all reported symptoms and concerns of participants and their caregivers. The study PI or a designated physician colleague will be available by pager and/or mobile phone 24/7 for any emergencies or urgent concerns about potential adverse events during the course of the study. Adverse events will be monitored and recorded at each study visit and with a phone call at week 2 after beginning the study intervention. Safety labs (complete blood count with differential, chemistries, liver function tests, and thyroid stimulating hormone) will be obtained at screening and again at the completion of the active treatment phase, and the data will be reviewed periodically by the DSMB as described below.

The minor risks associated with phlebotomy will be minimized through use of calming behavioral strategies, offering topical anesthetic prior to blood draw, and having the blood draw performed by professionals with extensive experience in working with individuals with ASD. The maximum amount of blood drawn at any visit will not exceed 2 ml/kg body weight. To minimize risk of psychological distress associated with routine assessment and answering questions about personal history, every effort will be made to provide a relaxed atmosphere with as little waiting time as possible; breaks will be offered as needed. Confidentiality will be protected by limiting identifiable personal information on source documents, which will be labeled with a designated participant number and initials only. Paper charts will be maintained in a locked file cabinet behind two locked doors and will only be accessed by staff directly involved in the study and monitors from regulatory agencies. Electronic data files will be protected by password and encryption, and only study staff directly involved in data collection/ entry and monitors from regulatory agencies will have access to them. Saliva specimens will be stored in a locked cabinet and labeled with a personal identifier number rather than patient name, and the key will be maintained in an encryption-protected digital file accessed only by study staff and regulatory monitors.

If a participant experiences significant adverse events that affect tolerability, may reasonably be related to taking the investigational product, and do not meet the protocol definition for a serious AE (including non-extreme behavioral symptoms), the dose may be reduced by approximately 30% according to the following dose modification schedule:

Weight category	Prescribed weight-based dose of glucoraphanin	Prescribed # of tablets	70% of weight-based dose (ie, 30% reduction)	Prescribed # tablets for reduced dose (rounded to nearest 15 mg increment)*
<100 lb	45 mg	3	31.5 mg	2
100-125 lb	75 mg	5	52.5 mg	3
126-175 lb	90 mg	6	63 mg	4
176-199 lb	105 mg	7	73.5 mg	5
≥ 200 lb	120 mg	8	84 mg	6

*Rounded down if equidistant between two 15 mg dosing increments

If the dose is reduced during the course of the study, the participant will continue the lowered dose for the remainder of the study. If adverse events do not improve within 14 days of lowering the dose, the investigational product may be permanently discontinued at the discretion of the treating study physician, participant, and participant's guardian. If a participant exhibits extreme exacerbation of behavioral symptoms (such as self-injurious behavior or aggression) that do not improve substantially following a dose reduction and are determined "probably" or "definitely" related to taking the investigational product, the investigational product may be permanently discontinued sooner than 14 days post-reduction, and the DSMB will be notified. As individuals with ASD often have cyclical changes in behavior independent of medication effects, the study staff will rely on caregivers' experience with the participant to determine if any observed behavior changes are within the expected range for that individual. If the investigational product is stopped permanently, the participant and his caregiver will be asked to complete the remainder of the study visits, return any unused study drug, and repeat safety lab studies.

There is some evidence from animal studies and in vitro studies suggesting that sulforaphane and/or its metabolites can inhibit expression of CYP 1A2, 3A4, and 2D6, which could be a potential source of drug-drug interactions if the participant is taking medications that are substrates of these enzymes. The risk of possible drug-drug interactions is not well-established. Upon reviewing the participants concurrent medications at screening and all subsequent visits, the study physician will consult an extensive P450 Table to identify possible drug-drug interactions. If the participant develops significant adverse effects that appear consistent with increased exposure to a concurrent medication metabolized by 1A2, 3A4, or 2D6 and do not improve with a 30% investigational product dose reduction within 14 days, discontinuing the investigational product may be recommended.

If a participant has a seizure while taking the study medication, the study medication will be stopped, and the study PI and DSMB will be notified. The participant will be evaluated by the study PI and his primary care physician, treated, and monitored appropriately. The participant and his caregiver will be asked to return to complete the remainder of the study visits, return any unused study drug, and repeat safety lab studies.

If the participant newly requires a long-term medication that is thought to interact adversely with the study medication, the study medication will be stopped. The participant and his caregiver will be asked to return to complete the remainder of the study visits, return any unused study drug, and repeat safety lab studies.

If a participant experiences a serious adverse event that is thought to be related to taking the study medication (as determined by an independent medical monitor, the study medication will be permanently discontinued, and the appropriate overseeing regulatory organizations will be notified (FDA, IRB, and DSMB). The participant and his caregiver will be asked to return to complete the remainder of the study visits, return any unused study drug, and repeat safety lab studies.

III. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

Preliminary evidence suggests that sulforaphane can improve social relatedness and have an overall calming effect for some individuals with ASD [1]. Treatment with sulforaphane may also improve mitochondrial dysfunction and redox imbalance, both of which have been implicated in autism. Broader benefits for the autism community include new knowledge about a safe and potentially efficacious treatment for core symptoms of autism, for which current treatment options are limited.

IV. IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

ASD is a neurodevelopmental disorder that is now estimated to affect 1 in 68 children in the U.S., including 1 in 42 boys [8], and leads to substantial, lifelong burden for individuals, families, and communities. Use of psychiatric medication in ASD is high with 66% of affected individuals taking at least 1 psychotropic medication by adolescence [9]. Faced with heavy side effect burden and often limited efficacy, caregivers frequently turn to supplements that promise low toxicity but lack rigorous investigation and oversight. To date, no pharmacological agents have indication for the treatment of core social communication impairments in autism, and safe, evidence-based, rationally-developed treatments for ASD are greatly needed. Sulforaphane has shown initial promise as a well-tolerated and potentially efficacious treatment for ASD in a small clinical trial [1], though this finding warrants replication before being adopted into clinical practice more widely. The form of sulforaphane used in the ASD study by Singh et al. [1] is also prohibitively expensive to manufacture on a large scale and no longer produced in the United States, and Avmacol© (Nutramax, Inc), a sulforaphane precursor, is a far less costly and more readily available alternative. It is not yet clear if a sulforaphane precursor (and converting enzyme) will have benefit similar to direct ingestion of sulforaphane, and the currently proposed study will contribute to knowledge about the efficacy and safety of this widely available nutraceutical for the treatment of ASD.

V. DATA AND SAFETY MONITORING PLAN

a. Definitions of Unanticipated Problems, Adverse Events, and Serious Adverse Events

1. Unanticipated Problems Involving Risk to Subjects or Others is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigator's brochure, etc.),
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research,
- Serious (as defined below) "***Serious" is different than "severe" as reported in the CTC criteria that applies a grade to the AE.***

2. An **adverse event (AE)** is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries will be regarded as adverse events. Abnormal results of diagnostic procedures (eg, laboratory values) are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

3. A **serious adverse event** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event (of major clinical significance and may require intervention to prevent one of the serious outcomes noted above)

All adverse events that do not meet any of the criteria for serious will be regarded as ***non-serious adverse events***.

b. Monitoring of adverse events: The study physician will review AEs with the participant and family member at each study visit (baseline, week 2, week 4, week 8, week 12, week 16), and patients and their families will have 24/7 access to the study PI or a designated covering physician by phone/ pager to report possible AEs as needed between visits. AEs will be recorded on an AE log and assigned a numerical grade of 0 (not at all a problem), 1 (mild problem), 2 (moderate problem), and 3 (severe problem). As previously noted, an AE rated as severe ("3") is distinct from classification of a **serious AE**, defined above. The study period during which adverse events must be reported is defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this

study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

A *preexisting condition* is a reported symptom, clinically significant physical exam finding, or laboratory finding that is present at the start of the study and will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period. At interval study visits and at the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will also be recorded and documented as an adverse event.

All unresolved adverse events will be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the study physician will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

c. Entity responsible for monitoring: The study PI or a designated Co-Investigator will be the primary individual responsible for eliciting, recording, and monitoring AEs, and the study PI will have the ultimate responsibility of responding to AEs and reporting serious AEs to monitoring agencies (e.g., IRB, DSMB, FDA) in a timely manner. Additional monitoring entities for this study will include the University of North Carolina IRB and the North Carolina Translational and Clinical Science (NC TraCS) Institute Data and Safety Monitoring Board (DSMB).

Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event (SAE), will be reported to the FDA within 7 calendar days using FDA Form 3500A for mandatory reporting. SAEs will also be reporting to the UNC IRB by the study PI via IRBIS (the IRB information system for UNC) as well as the NC TraCS DSMB within 7 calendar days of becoming aware of the adverse event. Significant new information about ongoing serious adverse events will be provided promptly to the FDA, UNC IRB, and DSMB.

The NC TraCS DSMB will consist of a chair, an ethicist, an epidemiologist, a biostatistician, and one or more clinical researchers, each serving three year terms (please see attached TraCS DSMC Charter for a listing of current Committee members). The DSMB will review all SAEs, which the study PI will submit to the DSMB within 7 calendar days of the event. The DSMB will also review safety information (baseline and end-of-treatment screening labs; adverse event forms; significant physical exam findings) after every 10 participants have completed the study. All data presented to the DSMB will be de-identified to protect the confidentiality of participants. Due to the relatively small size and anticipation of minimal risk associated with this study, no interim data analysis is planned. The DSMB will report their findings to the UNC Biomedical IRB as appropriate.

Though not expected, any of the following events will also be reported to the UNC IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor-investigator to modify the investigators brochure, protocol, or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.

- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example, safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the sponsor-investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the principal investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

VI. References

1. Singh, K., et al., *Sulforaphane treatment of autism spectrum disorder (ASD).* Proc Natl Acad Sci U S A, 2014. **111**(43): p. 15550-5.
2. Shapiro, T.A., et al., *Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study.* Nutr Cancer, 2006. **55**(1): p. 53-62.
3. Egner, P.A., et al., *Bioavailability of Sulforaphane from two broccoli sprout beverages: results of a short-term, cross-over clinical trial in Qidong, China.* Cancer Prev Res (Phila), 2011. **4**(3): p. 384-95.
4. Egner, P.A., et al., *Rapid and sustainable detoxication of airborne pollutants by broccoli sprout beverage: results of a randomized clinical trial in China.* Cancer Prev Res (Phila), 2014. **7**(8): p. 813-23.
5. Riedl, M.A., A. Saxon, and D. Diaz-Sanchez, *Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway.* Clin Immunol, 2009. **130**(3): p. 244-51.
6. Alumkal, J.J., et al., *A phase II study of sulforaphane-rich broccoli sprout extracts in men with recurrent prostate cancer.* Invest New Drugs, 2015. **33**(2): p. 480-9.
7. Cipolla, B.G., et al., *Effect of Sulforaphane in Men with Biochemical Recurrence after Radical Prostatectomy.* Cancer Prev Res (Phila), 2015.
8. *Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010.* MMWR Surveill Summ, 2014. **63**(2): p. 1-21.
9. Coury, D.L., et al., *Use of psychotropic medication in children and adolescents with autism spectrum disorders.* Pediatrics, 2012. **130 Suppl 2**: p. S69-76.

17 Appendix 6C: NC TraCS DSMB Charter

North Carolina Translational and Clinical Sciences (TraCS) Institute

Data Safety Monitoring Board (DSMB) Charter

Revised 10-19-2015

NC TraCS Institute

Data and Safety Monitoring Board Charter

Table of Contents

Abbreviations

AE	Adverse event
DSMB	Data and Safety Monitoring Board, NC TraCS Institute
DSMC	Lineberger Data and Safety Monitoring Committee, PRC
CTRC	Clinical and Translational Research Center
IRB	UNC Biomedical Institutional Review Board
NIH	National Institutes of Health
PI	Principal Investigator
PRC	UNC Lineberger Oncology Protocol Review Committee
TraCS	North Carolina Translational and Clinical Sciences Institute
UNC	University of North Carolina at Chapel Hill

Introduction

The North Carolina Translational and Clinical Sciences (TraCS) Institute is committed to the safety of patients participating in clinical trials at our institution. In addition, it is committed to data accuracy and protocol compliance. The NC TraCS Institute has established an institutional plan to provide data safety and monitoring for selected clinical trials conducted at UNC. This plan is designed to comply with policies and guidelines regarding data and safety monitoring from the National Institutes of Health (http://grants.nih.gov/grants/guide/notice-files/not98-084.html; http://nih.gov/grants/guide/notice-files/NOT-OD-00-038.html).

This charter is for the Data Safety and Monitoring Board (DSMB) of the North Carolina TraCS Institute. The charter contains the following:

- A description of the composition and organization of the DSMB
- Details the roles and responsibilities of DSMB members
- Outlines the responsibilities of the Principal Investigators and Sponsors
- Lists important contact persons

Organization

Overview

The DSMB functions as a committee within the NC TraCS Institute. The charge to the DSMB is review clinical trials to assure patient safety both by evaluating adverse events (AEs) and interim analyses of both safety and efficacy.

Committee Structure

The DSMB Chair (currently Dr. Ross Simpson) has the overall responsibility for the chairing the DSMB committee and reporting its findings to the UNC Biomedical IRB. The Committee will be under the direct supervision of the NC TraCS Institute Director or designated representative. The Committee will consist of a chair, an ethicist, an epidemiologist, a biostatistician, and one or more clinical researchers. Committee members will serve for 3-year terms, but may be reappointed. Members will be appointed to the Committee by the Chair, Director or designated representative. A member of the DSMB can be removed due to poor attendance, inadequate demonstration of effort, unprofessional conduct and /or failure to act in accordance with the objectives of the DSMB. A list of current DSMB members can be found in Appendix 1.

PROCESS

Eligibility

A clinical trial is defined as “a prospective study involving human subjects designed to answer specific questions about the effects or impact of particular biomedical or behavior interventions; these may include drugs, treatments, devices, or behavior or nutritional strategies.” Studies that include nutritional, behavioral, and psychosocial interventions are considered to be clinical trials. Studies evaluating diagnostics (imaging, etc.) in which findings alter the patient’s clinical care are also considered to be clinical trials. Observational studies, epidemiologic studies, studies of diagnostics that do not effect patient care, and studies that do not test interventions are not considered to be clinical trials.

DSMB Functions and Activities

The DSMB is responsible for reviewing data from clinical trials approved by the UNC Biomedical IRB. Investigators with commercially or governmentally sponsored trials will normally work with their sponsor’s DSMB. The DSMB will review data from the following types of trials, when review is deemed necessary by the Biomedical IRB or requested by the Principal Investigator (PI), in order to ensure the safety of participating subjects.

- Phase I, I/II, II, and II/III trials when such review is deemed necessary by the UNC Biomedical Institutional Review Board and the Principal Investigator.
- Phase III clinical trials (single site trials where the PI is a UNC faculty member and for which UNC is the sponsor)

- Phase IV clinical trials (single site trials where the PI is a UNC faculty member and for which UNC is the sponsor)
- Select multicenter clinical trials in which UNC is the coordinating center or the PI of the study is a UNC faculty member IF the DSMB determines that it has adequate resources to conduct the monitoring required of the study.

The DSMB will meet at least every other month and has the following responsibilities.
- Assessing risk and complexity of clinical trials submitted for review
- Determining the appropriate level of data and safety monitoring
- Reviewing serious adverse events (AEs) reports when requested.
- Reviewing yearly data and safety monitoring reports when requested. Recommending appropriate actions (closure, increased monitoring, etc.) to the Principal Investigator, UNC Biomedical IRB, and the Director or designated representative.
- Communicating its finding with the Biomedical IRB
- Preparing minutes for all meetings
- Preparing an annual summary of activity for review by the Director, NC TraCS Institute
- Review of the monthly reports of the UNC Lineberger Oncology Protocol Review Committee (PRC) -Data and Safety Monitoring Subcommittee (DSMS)
- Assessing ethical and scientific soundness of trials reviewed

DSMB-Investigator Communications
The DSMB will make available to investigators the following:
- An Annual Data Reporting form for annual reporting of all AEs
- A Data Safety Monitoring Plan template

Principal Investigators shall make available to the DSMB the following information:
- A copy of the approval letter from the UNC Biomedical Institutional Review Board
- A Data Safety Monitoring Plan
- All adverse event reports using the most current UNC Biomedical Institutional Review Board form
- An annual summary of all AEs

Relation to Biomedical Institutional Review Board and Lineberger Oncology Protocol Review Committee
The DSMB will report its findings and make recommendations to the UNC Biomedical IRB. For research conducted by the Lineberger Cancer Center, the DSMB will report its findings and make recommendations to the UNC Lineberger Oncology Protocol Review Committee (PRC). Trials required to have an independent data and safety monitoring board as defined in this charter are expected to use the NC TraCS Institute DSMB instead of establishing their own independent board, unless outside expertise not available on the DSMB is necessary to adequately monitor the trial.

Notice of a recommendation of early closure or suspension will be reported directly to the Biomedical IRB and the Director or designated representative. As appropriate, such recommendations will be reported to the Director of the Lineberger Cancer Center and the Director of the Clinical Translational Research Center. The Chair of the DSMB in conjunction with the Chair of the Biomedical IRB is responsible for seeing that these trials are closed to accrual.

Guidelines for Members
In order for the DSMB to fulfill its responsibilities, the member will observe the following guidelines:
- Members are free of apparent conflicts of interest involving financial, scientific, or regulatory matters. In case of any question of conflict of interest, standards used by NIH in determining conflict of interest for advisory committee members and investigators shall apply
- Members should assess trial objectives and design in an unbiased way.
- Members are guided both by pre-specified study performance criteria, such as early stopping rules, and also by a masked and, if necessary, unmasked review of all data prior to making decisions
- The DSMB members will review data and pertinent procedures in order to be confident that the data on which the decisions are based are accurate and complete

- All decisions of the DSMB shall be independent

PROTOCOL REVIEW AND DATA MONITORING

Monitoring and Reporting Requirements
In collaboration with the Principal Investigator and study biostatistician, the DSMB will set data monitoring and reporting requirements before study enrollment begins. Members will review the interim analysis plan to ensure the analyses planned will provide necessary information for DSMB decisions. Members will participate in specification and review of all tables and specifications for data that they will review in accordance with timelines established by agreement with the Principal Investigator.

The DSMB will have face-to-face meeting(s) to review and discuss results of interim analysis. The DSMB may also have face-to-face meeting(s) as prompted by unplanned interim analyses deemed necessary because of safety concerns. The DSMB staff or chair will prepare minutes of each meeting within ten (10) working days of each meeting. These minutes will be circulated and approved at the next regularly scheduled meeting of the DSMB.

The Principal Investigator and study biostatistician will work with the DSMB for appropriate preparation of reports to be viewed at DSMB meetings and resolution of questions arising during data analysis. If requested by the DSMB, the Principal Investigator will turn over to the chairperson of the DSMB evidence of validation of all computer programs used to generate reports and analyses for each DSMB meeting. The documents will be made part of the minutes of each meeting. All formal reports will be circulated to the DSMB members no later than one week prior to each DSMB meeting.

Data Review by the DSMB
DSMB meetings will consist of an open and a closed session. The open session will provide a forum for exchange of information among DSMB members, Principal Investigator, and study biostatistician. During the open session, the Principal Investigator may present a brief summary report of the study progress, including enrollment rates, data collection, and data quality. There will be an opportunity for the Principal Investigator to ask advice from the DSMB on any matter concerning conduct of the trial.

Only members of the DSMB will attend the closed session. During this session, the DSMB will address issues regarding the following: 1) safety concerns, 2) efficacy concerns, 3) termination of the trial due to pre-specified stopping criteria, and 4) ethical concerns. The DSMB will be furnished with relevant information by the Principal Investigator to make these decisions. During this session the DSMB members may ask the Principal Investigator to provide them with data that is partially unmasked (i.e., treatment A or treatment B without revealing what treatment A and B represent) or completely unmasked (i.e., identify treatment group).

In addition to the open and closed sessions, the voting members of the DSMB may meet in an executive session at their discretion. The DSMB will:
- Review the protocol and any protocol amendments
- Review the interim analysis monitoring plan, make recommendations, and give approval
- Review interim analysis reports and meet as a group
- Communicate recommendations in writing to the Principal Investigator

If there are any concerns about reviewed material (such as safety, efficacy, ethics, and stopping rules), the DSMB will take appropriate action. This may involve a request for additional information, or a request for an early, unscheduled meeting of the DSMB with the Principal Investigator and study biostatistician. At each meeting that includes review of interim data, the DSMB will recommend one of the following actions to the Principal Investigator, Biomedical IRB, and when appropriate the NC TraCS Institute Director or designated representative.
- Continue the study according to the protocol
- Modify the study protocol. Modifications may include such items as changes in the inclusion/exclusion criteria, nature and frequency of safety monitoring, study procedures, study drug/intervention dosing, consent form changes, subject re-notification, and any other changes deemed necessary
- Discontinue one or more study arms

- Discontinue the study

The DSMB may request additional data, analyses, or meetings to address specific concerns. The DSMB may hold additional meetings without knowledge of the sponsor or Principal Investigator.

Release of DSMB Recommendations
The DSMB will refrain from revealing to the sponsor, Principal Investigator, or any other party information that would lead to compromising the integrity of the trial unless such a release is required to protect subject safety. In particular, the following guidelines will be followed with respect to the dissemination of the DSMB interim analysis results.

- Individual patient treatment assignments will not be revealed
- Individual center results will not be revealed
- The magnitude of treatment differences in efficacy will not be revealed
- Study results will not be communicated to investigators
- The DSMB will not make any public disclosures of its discussions and decisions
- The DSMB will complete a brief report documenting decisions and rationale behind its decisions. The report will be conveyed confidentially to the Principal Investigator and sponsor, who in turn, will forward it to appropriate regulatory agencies. A copy of the report will also be made available to the UNC Biomedical Institutional Review Board, in form that does not contain information that could compromise the integrity of the clinical trial.
- When appropriate a copy of the report will also be made available to the Director of the Lineberger Cancer Center and/or the Director of the Clinical Translational Research Center.
- All DSMB decisions will be deemed advisory to the Principal Investigator, UNC Biomedical Institutional Review Board, and the Director, NC TraCS Institute.

Appendix 1 NC TraCS Institute DSMB Members, Year 2013 - 2015

Member	Department/School	Email
Ross J. Simpson, Jr., MD, PhD (chair)	Medicine	rsimpson@med.unc.edu
David Weber, MD, MPH	Medicine	dweber@unch.unc.edu
David Couper, PhD	Biostatistics	david_couper@unc.edu
Marcia Van Riper, RN, PhD	School of Nursing	vanriper@email.unc.edu
Kenneth Ataga, MD	Medicine	kenneth_ataga@med.unc.edu
Marie Rape, RN, BSN	NC TraCS Institute	marie_rape@med.unc.edu
Gretchen Stuart, MD, MPH	Obstetrics/Gynecology	gstuart@med.unc.edu
Samantha Meltzer-Brody, MD, MPH	Psychiatry	samantha_meltzer-brody@med.unc.edu

18 Appendix 6D: Clinical Studies List

Other Clinical Studies with Oral and Topical Broccoli Sprout Extracts

Approved IND Applications:

1. Andrew Nel (UCLA) entitled: "Effect of broccoli sprout extracts on pulmonary function in volunteers exposed to diesel exhaust particles."
IND# 76,411

2. Robert H. Brown (Anesthesiology, Johns Hopkins University School of Medicine): NA_00011275, entitled: "The effect of broccoli sprout extract on asthma." Approved 3/14/2008 JHM-IRB5
IND# 79, 274

3. Kala Visvanathan (Oncology and Epidemiology, Johns Hopkins Bloomberg School of Public Health), entitled:"Effect of broccoli sprout extracts on breast cancer."
IND# 103,019

4. Yuesheng Zhang (Roswell Park Cancer Institute, Buffalo, NY), entitled: "A Phase I pre-cystectomy trial of broccoli extract in patients with advanced bladder cancer."
IND# 103,466

5. Paul Talalay (Pharmacology, Johns Hopkins University School of Medicine) NA_00018779, entitled: "Effects of topical application of sulforaphane-containing broccoli sprout extracts on radiation dermatitis during external beam radiation therapy for breast cancer."
IND# 104,164

6. Paul Talalay (Pharmacology, Johns Hopkins University School of Medicine) RPN 03-05-28-03, entitled: "Induction of phase 2 enzymes in skin by broccoli sprout extracts." Approved renewal 7/28/2008 JHM-IRB2.
Amendment to IND# 104,164

7. Paul Talalay (Pharmacology, Johns Hopkins University School of Medicine) NA_00004897, entitled: "Effects of topical application of sulforaphane-containing broccoli sprout extracts to human skin on the dose of UVB radiation required to produce erythema." Approved 10/11/2007 JHM-IRB2
Amendment to IND# 104,164

8. David W. Lin (Fred Hutchinson Cancer Center, Seattle, WA) entitled: "*In Vivo* effects of sulforaphane supplementation on human prostate."
IND# 105,321

9. Robert Wise (Johns Hopkins Asthma and Allergy Center), entitled: "Broccoli sprout extract for antioxidant protection in COPD."

IND# 109,233

10. Joshi Alumkal (Oregon Health Sciences University), entitled: "The effects of sulforaphane-rich broccoli sprout extracts in patients with biochemical recurrence of prostate cancer."
IND# 109,782

11. Jackilen Shannon (Department of Veterans Affairs Medical Center, Portland, OR), entitled: "Chemoprevention of prostate cancer, HDAC inhibition & DNA methylation."
IND# 111,736

12. Thomas W. Kensler (Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health), entitled: "Broccoli Sprout Extract for Protection Against Environmental Toxins."
IND# 112,038.

13. John M. Kirkwood (University of Pittsburgh Cancer Institute), entitled: "A Pilot Study Evaluation of Sulforaphane in Atypical Nevi—Precursor Lesions: Assessment of STAT1 and STAT3 Risk Markers of Melanoma."
IND# 112,691

14. Paul Talalay (Pharmacology, Johns Hopkins University School of Medicine) NA_00045538, entitled: "Bioavailability of Stabilized Sulforaphane (SF) from Broccoli Sprout Extracts (BSE)."
IND# 113,228

15. Andrew Zimmerman (Lurie Family Autism Center, Massachusetts General Hospital), entitled: "Broccoli Sprout Extract in Autism."
IND# 113,542

16. Li Tang (Roswell Park Cancer Institute, Buffalo, NY), entitled: "A Pilot Study of Broccoli Sprout Extract in Patients with Invasive Breast Cancer."
IND# 117,001

17. Paul Talalay (Pharmacology, Johns Hopkins University School of Medicine) NA_00081276, entitled: "Prevention of Alcohol Intolerance."
IND# 118,023

18. David Alberts (The University of Arizona Cancer Center), entitled: "Pilot study to evaluate the signaling pathway modulation demonstrated by topical sulforaphane by acutely exposing human skin to solar simulated light."
IND# 118,454

19. Paul Talalay (Pharmacology, Johns Hopkins University School of Medicine), entitled: "Prostaphane."
IND# 126,335

20. Andrew Zimmerman (University of Massachusetts Medical School), entitled: “Sulforaphane treatment of children with autism spectrum disorder (ASD).”
IND# 127,062

21. Hua Jin (University of California, San Diego), entitled: “A 6-month randomized placebo controlled study to evaluate sulforaphane add-on effects in treatment of first onset and early stage schizophrenia.”
IND# 127,513

22. Robert Wood (Johnson Medical School, Rutgers University), entitled: “Sulforaphane in autism. A treatment trial to confirm phenotypic improvement with sulforaphane in individuals with autism.”
IND# 127,543

Johns Hopkins Medical Institutions IRB Approved Trials (Without IND Approval):

1. RPN 04-05-18-01, entitled: "Evaluation of the biological effects of broccoli sprout extract in smokers." Initial approval 10/25/2005 (P.I. J. Brahmer)

2. RPN 04-07-30-01, entitled: "Quantification of sulforaphane and sulforaphane metabolites in human prostate tissue after oral administration of broccoli sprout extracts." Initial approval 10/20/2004 (P.I. A.W. Partin)

3. NA_00035554, entitled: "Broccoli sprout extract (BSE) taste test." Approved 6/10/2010 (P.I. J.W. Fahey)

4. NA_00085573, entitled: "Conversion of Broccoli Sprout Glucosinolates to Isothiocyanates." Initial approval 8/1/2013 (P.I. P. Talalay)

5. NA_00008645, entitled: "Gastrointestinal conversion of broccoli sprout glucosinolates to isothiocyanates." Approved 10/25/07 (P.I. P. Talalay)

6. NA_00088662, entitled: "Conversion of Broccoli Seed Glucosinolates to Isothiocyanates- Dietary Supplements." Initial approval 12/5/2013 (P.I. P. Talalay)

7. NA_00009109, entitled: "Dietary intervention to ameliorate *Helicobacter pylori* infection in a high risk population." Approved 12/15/08 (P.I. J.W. Fahey)

8. H.18.02.03.11.81, entitled: "Chemopreventive Efficacy of Broccoli Sprouts, and Cross-Over Broccoli Sprouts Trial-Qidong. Study of the effects of broccoli sprout extracts on the urinary excretion of aflatoxin N^7-guanine adducts and phenanthrene tetraol levels." Approved 3/10/10 (P.I. T. W. Kensler)

9. IRB00077442, entitled: "Bioavailability of Sulforaphane from a Glucoraphanin-Rich Broccoli Supplement (Avmacol)." Initial approval 9/22/2015 (P.I. J. W. Fahey)

Clinical Trials of Broccoli Sprout Extract Registered in www.ClinicalTrials.gov

1. NCT00252018. The Effect of Broccoli Sprouts as a Nutritional Supplement in the Prevention of Cardiovascular Disease. [C. Torp-Pedersen, Department of Cardiology, Bispebjerg Hospital, Denmark] *The purpose of this study is to investigate whether a daily intake of dried broccoli sprouts will improve the endothelial function of the participants as measured by Flow mediated dilation (FMD). The dried sprouts are chosen because broccoli sprouts are known as containing large amounts of the glucosinolate glucoraphanin which in vitro and in animal models has been shown to have a positive effect on the endothelium as measured by NO release.*
Status: Completed

2. NCT00255775. Broccoli Sprout Extract in Preventing Lung Cancer in Smokers [J.Brahmer, Johns Hopkins.] *The purpose of this study is to investigate the effectiveness broccoli sprout extract has in preventing lung cancer in smokers.*
Status: Completed

3. NCT00607932. Brassica Vegetables or Indole-3-Carbinol in Treating Patients With PSA Recurrence After Surgery for Prostate Cancer [Vanderbilt University]. *Eating a diet high in vegetables may lower the risk of some types of cancer. Brassica vegetables (such as cabbages, kale, broccoli, Brussels sprouts, and cauliflower) and indole-3-carbinol (a substance found in cruciferous vegetables) may help lower the risk of prostate cancer recurrence. This randomized clinical trial is studying the side effects and how well Brassica vegetables work compared with indole-3-carbinol in treating patients with PSA recurrence after surgery for prostate cancer.*
Status: Completed

4. NCT00621309. Sulforaphane as an Antagonist to Human PXR-mediated Drug-drug Interactions. [D.L. Eaton, University of Washington, Seattle, WA.] *The purpose of this project is to determine if sulforaphane (SFN) can be used to block adverse DDIs that occur when drugs bind to and activate the PXR receptor and subsequently induce CYP3A4 activity. This project will determine whether SFN can prevent the drug Rifampin from binding to PXR and increasing CYP3A4 activity in humans following oral administration of SFN (broccoli sprout extract).*
Status: Unknown

5. NCT00836186. Cytokine Expression During Radiation for Breast Cancer [Sidney Kimmel Comprehensive Cancer Center]. *To assess the magnitude and frequency of changes in chemo/cytokine expression in women receiving radiation treatment. To asses the impact of race/ethnicity on the magnitude and frequency of changes in chemo/cytokine expression during radiation therapy for breast cancer. And finally to assess the interaction between radiation-induced chemo/cytokine expression changes, and race/ethnicity, with respect to normal tissue reactions to radiation and tumor-associated outcomes.*
Status: Unknown

6. NCT00843167. Broccoli Sprout Extract in Treating Women Who Have Had a Mammogram and Breast Biopsy. [J. Shannon, Knight Cancer Institute, Oregon Health and Science University, Portland, OR.] *This randomized phase II trial is studying how well broccoli sprout extract works in treating women with newly diagnosed ductal carcinoma in situ and/or atypical ductal hyperplasia.*

Status: Completed, has Results

7. NCT00882115. Broccoli Sprout Extract Effects on Allergic Inflammation in the Nose. [A. Nel, UCLA and NIAID]. *The purpose of this study is to explore the effects of broccoli sprout extract on the inflammatory process in the nose caused by diesel exhaust particles, which are present in air pollution.*
Status: Completed

8. NCT00894712. Topical Application of Sulforaphane-containing Broccoli Sprout Extracts on Radiation Dermatitis. [R.Zellars and P. Talalay, Johns Hopkins] *The purpose of this study is to investigate the protective effects of topical sulforaphane-containing broccoli-sprout extracts (BSE) on radiation-induced dermatitis in women undergoing external-beam radiation therapy for breast cancer. This investigation will employ ionizing rather than ultraviolet radiation. The objective is to determine and quantify the effect of topical BSE on radiation-induced skin erythema.*
Status: Active, Not Recruiting

9. NCT00946309. Effects of Sulforaphane on Normal Prostate Tissue. [D.W. Lin, Fred Hutchinson Cancer Research Center, Seattle, WA] *The purpose of this study is to identify the biological effects of a high-sulforaphane broccoli sprout extract in normal prostate tissue. The investigators hypothesize that consumption of high-sulforaphane broccoli sprout extract every other day will inhibit growth of prostate cancer cells.*
Status: Enrolling by Invitation

10. NCT00982319. Study to Evaluate the Effect of Sulforaphane in Broccoli Sprout Extract on Breast Tissue. [K. Visvanathan, Johns Hopkins.] *The purpose of this research is to examine the effect of a broccoli sprout preparation on specific factors in breast tissue that are related to breast cancer risk and to assess whether sulforaphane a key component of broccoli sprouts increases the levels of protective enzymes in breast tissue. In addition, the investigators will also examine how acceptable the broccoli sprouts preparation is to the study participants.*
Status: Completed

11. NCT00994604. The Effects of Broccoli Sprout Extract on Obstructive Lung Disease [R. B. Brown, Johns Hopkins.] *The purpose of this study is to examine whether broccoli sprout extract can effect lung function measurements in individuals with asthma and COPD.*
Status: Completed

12. NCT01008826. Cross-Over Broccoli Sprouts Trial. [T.W. Kensler, Johns Hopkins University.] *The study hypothesis tested is that broccoli sprouts are effective at altering the urinary levels of metabolites of the hepatocarcinogen aflatoxin B1 and of the air-borne pollutant phenanthrene in residents of Qidong, PRC, where exposures are unavoidable and high. The study will evaluate which of two formulations of broccoli sprouts beverage, glucoraphanin-rich*

or sulforaphane-rich, exhibits the best bioavailability and is most effective at modulating the biomarkers.
Status: Completed

13. NCT01108003. Broccoli Sprout Extract in Treating Patients With Transitional Cell Bladder Cancer Undergoing Surgery. [Y. Zhang, R. Pili, Roswell Park Cancer Center, Buffalo, NY]. *This phase I trial is studying the side effects of broccoli sprout extract in treating patients with transitional cell bladder cancer undergoing surgery.*
Status: Terminated

14. NCT01114399. Diet and Vascular Health Study. [Institute of Food Research, Norwich, Norfolk, United Kingdom]. *The aim of this study is to examine the effects of a diet rich in broccoli on cardiovascular disease risk using biochemical indicators such as blood lipid profiles, most notably cholesterol; markers of inflammation as well as established physiological measurements such as Pulse wave velocity (PWV), Augmentation index (AIx) and Ambulatory Blood Pressure Measurements (ABPM). Broccoli contains compounds known as glucosinolates which are metabolised to isothiocyanates when consumed. The major glucosinolate in broccoli is known as glucoraphanin which produces the isothiocyanate sulforaphane. The glucosinolates are thought to be the principal component in broccoli that may reduce CVD risk. The investigators will use a standard cultivar of broccoli and a cultivar that has enhanced levels of glucosinolates ('HG broccoli'). This broccoli has been used in previous intervention studies (e.g. ClinicalTrials.gov NCT00535977). Volunteers will be asked to consume 400g of standard broccoli, HG broccoli or peas each week over a 12 week period in a double blinded (for the broccoli) parallel study. The volunteers recruited will, according to the Joint British Societies (JBS 2) Guidelines on the prevention of cardiovascular disease (CVD) in clinical practise, have a 10-20% (mild to moderate) risk of developing cardiovascular disease or having a cardiovascular (CV) event in the next 10 years.*
Status: Completed

15. NCT01183923. Dietary Interventions in Asthma Treatment: Sprouts Study. [E. Matsui, Johns Hopkins University]. *The main objectives are to test the effect of broccoli sprouts (BS) on biomarkers of oxidative stress (OS), inflammation, basophil activation, and clinical outcomes in mouse allergen-induced asthma by: (1) determining if BS improves lung function and airways symptom responses in mouse-sensitized adults with asthma undergoing environmental mouse allergen challenge (EMAC), (2) examining the effect of BS on OS, inflammation, and basophil activation, and (3) examining the effect of BS on changes in OS, inflammation, and basophil activation after EMAC.*
Status: Suspended

16. NCT01228084. Sulforaphane in Treating Patients with Recurrent Prostate Cancer. [J. Alumkal, OHSU Knight Cancer Institute, Portland, OR]. *This phase II trial studies how well sulforaphane works in treating patients with recurrent prostate cancer. The primary objective is to determine the proportion of patients who achieve a 50% decline in prostate-specific antigen (PSA) levels within 20 weeks of sulforaphane treatment.*
Status: Completed, has Results

17. NCT01265953. Chemoprevention of Prostate Cancer, HDAC Inhibition and DNA Methylation. [Jackilen Shannon, Portland VA Medical Center, Portland, OR]. *The objective of the study is to identify mechanisms by which compounds found in cruciferous vegetables alter gene expression via epigenetic modifications (changes in gene expression) and may prevent prostate cancer development.*
The investigators have found that sulforaphane (SFN), an isothiocyanate found in cruciferous vegetables, inhibits histone deacetylase (HDAC) activity in human colorectal and prostate cancer cells.
Status: Unknown

18. NCT01129466. Effects of 2 Different Broccoli Sprout Containing Supplements on Nasal Cells in Healthy Volunteers (Broccosprout). [T. Noah, University of North Carolina, Chapel Hill, NC]. *To compare the change in nasal cell HO-1 expression induced by two different preparations of sulforaphane (SFN)-containing nutritional supplements. In this pilot study, subjects will be randomized to receive either the equivalent of 4 ounces/day of fresh broccoli sprouts in food and tea, or the equivalent amount as broccoli sprout homogenates, for 3 consecutive days. Nasal lavage and blood samples will be obtained before, during and after this 3-day period. After at least a 2-week washout interval, the protocol will be repeated with the alternate supplement, in a crossover design. The primary analysis will test the hypothesis that broccoli sprouts in food will increase HO-1 expression to a similar degree as the equivalent amount of sprouts in homogenates.*
Status: Completed

19. NCT01269723. Effects of Sulforaphane (SFN) on Immune Response to Live Attenuated Influenza Virus in Smokers and Nonsmokers. [T. Noah, University of North Carolina, Chapel Hill, NC]. *The purpose is to learn about short term responses to live attenuated influenza virus (LAIV, administered via Flumist® vaccine) between smoking and nonsmoking volunteers treated with broccoli sprout homogenates ("shake") or a placebo homogenate. Subjects will be seen for a screening visit (inc. HIV test) and then randomly assigned to receive 1 of 2 homogenates. Nasal lavage (NL), blood samples and nasal biopsies will performed prior to and after study interventions.*
Status: Completed

20. NCT01315665. Effect of Sulforaphane in Broccoli Sprouts on Nrf2 Activation. [JF Chmiel, Rainbow Babies and Children's Hospital, Cleveland, OH]. *The purpose of this study is to investigate the effect of sulforaphane from macerated broccoli sprouts in humans and to evaluate less invasive methods of assessing potential anti-inflammatory drugs in cystic fibrosis.*
Status: Completed

21. NCT01335971. Broccoli Sprout Extracts Trial (BEST). [R. Wise, Johns Hopkins University]. *This study will investigate whether ingestion of sulforaphane by chronic obstructive pulmonary disease (COPD) patients increases Nrf2 activity and expression of downstream antioxidants in alveolar macrophages and bronchial epithelial cells? Accordingly, the investigators are conducting a placebo-controlled randomized proof of principle trial of two oral doses of sulforaphane, 25 and 150 micromoles, for 4 weeks in 90 COPD patients. Collections of alveolar macrophages by BAL, bronchial epithelial cells by endobronchial brushings will be performed at baseline and 4 weeks. Other biospecimens will include nasal epithelial cells, PBMCs, and expired breath condensate. The goal is to*

establish a safe and tolerable dose of sulforaphane that effects in vivo antioxidants via Nrf2, in order to have a novel candidate treatment for longer-term efficacy trials.
Status: Unknown

22. NCT01344330. Cruciferous Vegetable Intake and Histone Status in Screening Colonoscopy Patients [Texas A&M University]. *This research study will assess cruciferous vegetable intake in patients presenting for screening colonoscopy and correlate intake with histone status and histone deacetylace (HDAC) expression in tissue biopsy specimens and peripheral blood mononuclear cells (PBMCs). The investigators will also measure sulforaphane (SFN) metabolites in blood as a biomarker of cruciferous vegetable intake.*
Status: Recruiting

23. NCT01357070. Effect of Broccoli Sprout on Blood Levels of Sulforaphane to Reduce Responsiveness of Immune System. [PC Evans, Imperial College London, UK]. *With particular attention to its role in reducing atherosclerosis, investigators want to test whether the consumption of a brocco-sprout smoothie containing sulforaphane can protect white blood cells from becoming activated in the presence of an experimental stress and how long this protective effect lasts for. To do this, the investigators will be analyzing inflammatory changes in blood samples taken at different times during the study.*
Status: Unknown

24. NCT01437501. Broccoli Sprout Intervention in Qidong, P.R. China. [Johns Hopkins Bloomberg School of Public Health]. *This study is a 12 week placebo-controlled Phase II broccoli sprout intervention to be conducted in Qidong, P.R. China. One thousand two hundred people from the farming townships will be screened and three hundred eligible individuals will be enrolled in the study. Participants will be randomized into two treatment groups: one will receive a juice beverage containing glucoraphanin- and sulforaphane-rich broccoli sprout extract, pineapple juice, lime juice, and water and the other will receive a placebo beverage containing pineapple juice, lime juice and water. Participants will drink their assigned beverage every evening and provide biweekly urine samples and monthly blood samples. The principal endpoints of this study are pharmacokinetic evaluation of elimination of glucoraphanin/sulforaphane and their metabolites in urine and pharmacodynamic evaluation through measures of urinary levels of exposure biomarkers for dietary and air-borne toxins, which are known to be high in this population.*
Status: Completed, has Results

25. NCT01474993. Sulforphane-rich Broccoli Sprout Extract for Autism. [Lurie Autism Center, Cambridge, MA]. *The primary objectives of this study are to answer whether there is evidence of measurable effects on social responsiveness (primary outcome) and other behavioral symptoms after treatment of autistic male adolescents and adults with orally administered sulforaphane-rich Broccoli Sprout Extract (efficacy). The secondary objectives of this study are to answer whether treatment of male adolescents and adults with autism using orally administered sulforaphane-rich Broccoli Sprout Extract within a specified dose range is safe (toxicity); treatment with sulforaphane-rich Broccoli Sprout Extract is well tolerated (side effects and adverse events); key cellular biomarkers support the hypothesized mechanisms (proof of principle).*
Status: Completed, has Results

26. NCT01522703. Effects of Whole Sprouts on Upper Airway Allergic Inflammation [Johns Hopkins University]. *The primary objective of this study is to determine if broccoli sprouts (BS) improves nasal inflammatory, oxidative stress (OS), and symptom responses to nasal mouse allergen challenge (NAC) in mouse-sensitized adults perennial allergic rhinitis.The study is a double-blind, placebo-controlled, randomized trial to compare BS to placebo in 24 mouse-sensitized adults with asthma or perennial rhinitis and who develop rhinitis symptoms with nasal mouse allergen challenge. Twenty-four adults (age 18-50) who meet these eligibility criteria will be randomized to receive either: (a) BS or (b) placebo. At the baseline NAC, FENO, FEV1, nasal epithelial gene expression, urinary OS biomarkers, serum inflammatory and OS biomarkers, basophil activation, and symptoms will be assessed both before and after NAC to establish pre-intervention responses to NAC. Subjects will eat a sprouts sandwich daily for three days, and then undergo repeat NAC that includes measurement of the above outcomes both before and after NAC.*
Status: Completed

27. NCT01543074. Dietary Histone Deacetylase Inhibitors (HDAC). [Oregon State University]. *This pilot, three-week study will help scientists understand more about how the foods people eat can modify histone deacetylases, enzymes the body produces naturally. Broccoli sprout extract and garlic oil are thought to modify these enzymes. The purpose of this study is to see if taking broccoli sprout extract alone, garlic oil alone, or broccoli sprout extract and garlic oil together, can decrease the action of histone deacetylase (HDAC) and turn on genes in white blood cells. There will be 80 people in this study.*
Status: Terminated, has Results

28. NCT01568996. Pilot Study Evaluating Sulforaphane in Atypical Nevi-Precursor Lesions. [University of Pittsburg]. *This is a pilot study to see if oral administration of freeze dried, powdered broccoli sprouts have any effect on whether moles end up becoming melanoma.*
Status: Recruiting

29. NCT01715480. Effect of Broccoli Sprouts Homogenate on SS RBC. [J.-T. A. Chi & M. J. Telen, Duke University]. *The overall purpose of this study is to obtain a better understanding of the biological response of red blood cells to sulforaphane contained in fresh broccoli sprouts that have been put through a blending process. This study will use commercially available fresh broccoli sprouts certified by Brassica Protection Products LLC (BroccoSprouts®). This product can also be purchased at some local grocery stores in the produce section. It is believed that NRF2, a transcription factor encoded by the NFE2L2 gene, plays a role in the regulation of defense against oxidative stress. The detrimental accelerated breakdown of sickle cell disease (SCD) red blood cells (SS RBC) is partially due to reduced anti-oxidative capacity. Previous analysis of SS RBC microRNAs revealed that a reduced level of NRF2, the master regulator of anti-oxidative stress capacity, contributes to reduced resistance to oxidative stress and increased hemolysis; NRF2 also induces fetal hemoglobin (HbF), which is known to prevent SS RBC sickling.*
First, erythroid progenitors from normal and SCD subjects will be tested ex-vivo to find out how sulforaphane, a natural NRF2 activator, affects the oxidative stress capacity, HbF expression, and microRNA expression of red cells.
Second, a pilot clinical trial will be conducted to determine the safety and physiological effects of 3 weeks of daily consumption of broccoli sprout homogenate in a cohort of Hb SS/SB0 thalassemia

adult SCD patients. During this study, subjects RBCs will be assayed for changes in anti-oxidative stress capacity and microRNA composition in mature SCD red blood cells.
Status: Completed

30. NCT01625130. Effect of Sulforaphane-rich Broccoli Sprout Homogenate on Ozone Induced Inflammation Through Modulation of NRF2 (BroccOz). [M. Hernandez, University of North Carolina, Chapel Hill]. *Purpose: To determine if modulation of NRF2 with a Sulforaphane enriched supplement modifies responses to O3. Participants: Recruitment of up to 70 healthy volunteers, ages 18-50, for completion of 36 volunteers. Procedures: This is a randomized, placebo controlled 2x2 crossover study of treatment with an NRF2 modifier versus placebo in healthy volunteers which will examine airway inflammation before and 4 hours after a 2 hour 0.4 ppm O3 exposure. Participants will be randomized to received either the NRF2 modifier, SFN oral supplement (i.e. broccoli sprout shake), or placebo (alfalfa shake) for 3 days followed by a 0.4 ppm O3 exposure for 2 hours. At least 2 weeks later subjects will return for a 2nd supplementation treatment (using the alternative supplement to that provided initially) followed by an ozone exposure identical to the initial one.*
Status: Completed

31. NCT01726127. Green Vegetables and Women's Health. [S. A. Tanumihardjo & N. Binkley, University of Wisconsin, Madison]. *The 2010 Dietary Guidelines for Americans recommend that individuals consume 4.5 to 5 cups fruits and vegetables daily. However, at current intake levels, fruit consumption will have to improve by more than 100% and vegetable intake by 50% to meet this recommendation. Importantly, intake of brightly colored fruits and vegetables is even lower when potatoes are not considered. It is possible that improved fruit and vegetable intake will have beneficial health effects. For example, higher intakes of fruits and vegetables, and particularly cruciferous vegetables (e.g., broccoli, Brussels sprouts, cauliflower, etc.), are associated with lower rates of many degenerative diseases, including some cancers, yet this group of vegetables may continue to be under-consumed due to their strong flavors. A supplement made from these vegetables (Cruciferous CompleteTM made by Standard Process Inc. Palmyra, WI) contains a group of phytochemicals called glucosinolates that can shift estrogen metabolism in a favorable way. One proposed biomarker of chemoprotection from breast cancer is the urinary estrogen metabolite ratio of 2- to 16α-hydroxyestrogens (2:16). In the main study, the effects of cruciferous vegetables (broccoli or Brussels sprouts), Cruciferous CompleteTM whole food supplements, or placebos on this ratio of urinary estrogen metabolites in healthy premenopausal women will be compared over an eight-week period. The investigators hypothesize that treatment with daily supplements will increase the 2:16 ratio as compared to daily consumption of a combination of Brussels sprouts and broccoli or a placebo, suggesting reduced breast cancer risk.*
In a sub-study, the relationships between serum α-carotene, β-carotene, β-cryptoxanthin, lutein and lycopene with dietary carotenoid intake as measured by a food frequency questionnaire and body composition will be evaluated in healthy premenopausal women. Carotenoids are a family of lipophilic compounds found primarily in colorful plant tissues and their concentration in human blood reflects dietary intake of carotenoid-rich foods. Carotenoid levels in the blood of healthy women do not appear to be influenced by menstrual status, but are inversely associated with body fatness. Thus, serum carotenoid concentrations may serve as a functional marker for chronic disease risk associated with excess body fat.
Status: Completed

32. NCT01753908. Broccoli Sprout Extract in Treating Patients With Breast Cancer [Roswell Park Cancer Institute]. *This randomized pilot trial studies broccoli sprout extract in treating patients with estrogen receptor-positive breast cancer. Broccoli sprout extract contains ingredients that may prevent or slow the growth of certain cancers.*
Status: Recruiting

33. NCT01716858. An Open Study of Sulforaphane-rich Broccoli Sprout Extract in Patients with Schizophrenia. [Chiba University, Japan]. *Accumulating evidence suggests a role of oxidative stress in the pathophysiology of schizophrenia. The potent antioxidant sulforaphane (SFN) is an organosulfur compound derived from a glucosinolate precursor found in cruciferous vegetables such as broccoli, Brussels sprouts and cabbage. The protection afforded by SFN is thought to be mediated via activation of the NF-E2-related factor-2 (Nrf2) pathway and subsequent up-regulation of phase II detoxification enzymes and antioxidant proteins, through an enhancer sequence referred to as the electrophilic responsive element or antioxidant responsive element. Recently, we reported that SFN could attenuate behavioral abnormalities in mice after the NMDA receptor antagonist phencyclidine. Considering the potent antioxidant effects of SFN, we have a hypothesis that SFN would be a potential therapeutic drug for schizophrenia. The purpose of this study is to determine whether SFN-rich broccoli sprout extract have beneficial effects in patients with schizophrenia.*
Status: Completed

34. NCT01743924. Bioavailability of Chemopreventive and Nutritional Compounds in Broccoli.[M. C. M. Sardiña, Universidad Politécnica de Cartagena, Murcia, Spain]. *The aim of this study was to determine the absorption of isothiocyanates (ITC) after ingestion of kailan-hybrid broccoli through the analysis of the correspondent urinary biomarkers. Furthermore, the effect of cooking (microwave) on the mentioned metabolic fate of these ITC was studied comparing to the uncooked vegetable.*
Status: Completed

35. NCT01845220. Prevention of Alcohol Intolerance. [Johns Hopkins University]. *This study is designed to determine whether Asians who are especiallly sensitive to alcohol exposure can be protected by boosting their activities of an alcohol disposing enzyme. This will be accomplished by administering broccoli sprouts that are rich in an agent that increases protective enzyme activity. The test system involves applying alcohol patches to the skin and measuring skin redness.*
Status: Active, Not Recruiting

36. NCT01845493. Sulforaphane Supplementation in Atopic Asthmatics. [M. L. Hernandez, University of North Carolina, Chapel Hill]. *The investigators will perform a pilot study of daily treatment with oral sulforaphane (SFN) for 3 days to determine if Nuclear factor (erythroid-derived 2)-like 2 (NRF2) induction is possible with this supplementation regimen in individuals with allergic asthma.*
Status: Completed

37. NCT01879878. Pilot Study Evaluating Broccoli Sprouts in Advanced Pancreatic Cancer [POUDER Trial]. [I. Herr, Heidelberg University, Germany]. *The goal of the POUDER trial is to determine the feasibility of a randomized controlled trial regarding the application of freeze-*

dried broccoli sprouts rich in sulforaphane and quercetin in patients with advanced pancreatic ductal adenocarcinoma that receive palliative chemotherapy.
Status: Recruiting

38. NCT01927666. The Conversion of ENcapsulated GlucorAphanin, Gut Microbiota Phylogeny and gEnotype Study (ENGAGE). [R. Mithen, Institute of Food Research, Norwich, Norfolk, United Kingdom]. *The variation in extent of isothiocyanate (ITC) excretion in urine from a capsule delivered dose of glucoraphanin will correlate with differences in (a) the gut microbiota, and (b) the genotype of key polymorphic genes (GSTM1, GSTT1, and other as yet undetermined candidate genes).*
Our study is a human dietary intervention in which participants will consume one capsule containing 100mg purified glucoraphanin from broccoli. The levels of glucoraphanin delivered by the capsule are similar to one to two portions of broccoli. As this is purified glucoraphanin there is no myrosinase enzyme present. All conversion of the glucoraphanin, contained within the capsule, to ITC will therefore occur by enzymes found in the gut microbiota.
The ability of the glucoraphanin in the capsule to be metabolised to ITCs by the gut microflora is unknown and will be assessed by measuring ITCs excreted in the urine. The ITCs will be quantified in urine using validated analytical methods.
It has been shown in human dietary intervention studies that the extent of conversion of glucosinolates varies greatly. In order to assess possible causative factors for variation in rate of glucoraphanin metabolism each participant will provide a faecal sample from which their faecal gut microbiota phylogeny will be analysed.
For a small number of participants a second faecal sample will be requested (a maximum of 3 participants). It is our aim to select one low, one medium and one high ITC excreter. Ideally the low and high excreters would be within the lowest and highest 5% excretion of ITC and the third participant would be as close to the mean ITC excretion as possible. The aim would be to culture the faecal microbiota over time with repeat dosing of glucoraphanin in order to select for microbiota that are able to metabolise glucoraphanin. It is known that the main hydrolysis product of glucoraphanin, sulforaphane, has a variety of benefits to human health, however there is no known clinical relevance to being a high, medium or low excreter of ITC.
For each participant a blood sample will also be requested in order that we can assess whether genotype affects the rate of ITC excretion in urine. The GSTM1 genotype and other, as yet, unidentified candidate genes of each participant will be determined. Whether the genotype affects the rate of ITC excretion either alone or in combination with the phylogenetic profile will be assessed.
Status: Active, Not Recruiting

39. NCT01948362. Sulforadex in Healthy Volunteers SAD. [J. Täube, Evgen Pharma, London, United Kingdom]. *To determine the safety and tolerability of single escalating doses of Sulforadex® in healthy male volunteers.*
Status: Completed

40. NCT01950143. Effect of Sulforaphane on Prostate CAncer PrEvention. [R. Mithen, Institute of Food Research, Norwich, United Kingdom,]. *The biology of prostate cancer is associated with changes in genes and metabolites within prostate tissue. There is robust evidence to suggest that a diet rich in broccoli can prevent or retard the development of prostate cancer by influencing these*

changes. This is likely to be due to a natural chemical that is obtained in these vegetables known as sulforaphane.
In this study, we are seeking to provide further evidence that a diet rich in broccoli may prevent prostate cancer from developing, and to understand how this may happen. We propose to undertake a human intervention study to test the hypothesis that a broccoli-rich diet can alter the metabolism and gene expression within prostate tissue of men under active surveillance in a manner that would reduce the probability of the emergence and progression of aggressive cancerous clones. Participants recruited onto this study will be randomly allocated to one of three dietary groups in which they will be required to consume one portion per week of a broccoli soup delivering a different concentration of sulforaphane. This will be part of their normal diet for one year.
Blood, urine and prostate biopsy tissue will be obtained before and after a 12 month intervention period. Prostate biopsies will be obtained either though transperineal template biopsies, a technique accepted as best clinical practice because it provides better sampling of the prostate, or transrectal ultrasound guided biopsy which is currently the standard of care for obtaining biopsies.
Status: Enrolling by Invitation

41. NCT02023931. Broccoli Sprout Extracts in Healthy Volunteers: A Pilot Study of Nrf2 Pathway Modulation in Oral Mucosa (BSE) [University of Pittsburgh]. *A pilot study has been designed to determine (primary objective) if three brief interventions with three oral BSE regimens will alter mRNA biomarkers of Nrf2 pathway signaling, including NQO1, GSTs and AKRs, in the oral mucosa of healthy subjects. Quantitative distribution data and preliminary effect size for specific Nrf2 targets, as measured in serial buccal cell scrapings, will be determined during the course of 3-day exposures to three BSE regimens. These data will inform the design of a randomized, phase II chemoprevention trial in patients with HPV-negative HNSCC. Ten healthy volunteers will be recruited for this pilot study, Age ≥ 18 years, both male and female: 1) The non-cancer population presenting to the University of Pittsburgh Eye and Ear Institute or the Hillman Cancer Center. This may include patients with benign disease or their friends/family members, or friends/family members of patients with cancer; 2) Professionals, staff, or students at the University of Pittsburgh.*
Status: Completed

42. NCT02055716. Sulforadex in Healthy Human Males MAD. [J. Täubel, Evgen Pharma, London, United Kingdom]. *To determine the safety and tolerability of multiple doses of Sulforadex® in healthy male volunteers over 7 days with qd or bid dosing*.
Status: Completed

43. NCT02300324. The Bioavailability Of Sulforaphane From Broccoli Soups Study (BOBS). [R. Mithen, Institute of Food Research, Norwich, Norfolk, United Kingdom]. *There is current evidence that suggests eating cruciferous vegetables like broccoli, cauliflower, cabbage is beneficial to our health as they contain compounds which are thought to reduce the risk of diseases such as cancer and cardiovascular diseases. Cruciferous vegetables are able to deliver in our body a group of compounds called isothiocyanates (ITCs) that are thought to be responsible of their health-promoting effects. Sulforaphane(SF) from broccoli is one of the most studied ITCs and its anticancer properties have been extensively investigated in in vitro and in vivo models.*
The investigators propose to undertake an intervention study to measure the bioavailability of SF from the soups used in another intervention study called ESCAPE. The investigators would like to investigate the rate and extent to which SF reaches the systemic circulation and is excreted in urine

by measuring SF and its metabolites in plasma and urine samples collected from apparently healthy participants after consumption of the three types of broccoli + stilton soups. The three types of soups are standard broccoli + stilton soups and two high-glucoraphanin (SF precursor) broccoli + stilton soups which are able to deliver different levels of SF.
Status: Completed

44. NCT02336087. Gemcitabine Hydrochloride, Paclitaxel Albumin-Stabilized Nanoparticle Formulation, Metformin Hydrochloride, and a Standardized Dietary Supplement in Treating Patients With Metastatic Pancreatic Cancer. [V. Chung, City of Hope Medical Center, Duarte, CA]. *This pilot phase I trial studies the side effects of gemcitabine hydrochloride, paclitaxel albumin-stabilized nanoparticle formulation, metformin hydrochloride, and a standardized dietary supplement in treating patients with pancreatic cancer that has spread from where it started to other places in the body. Drugs used in chemotherapy, such as gemcitabine hydrochloride and paclitaxel albumin-stabilized nanoparticle formulation, work in different ways to stop the growth of tumor cells, either by killing the cells, by stopping them from dividing, or by stopping them from spreading. Metformin hydrochloride, used for diabetes, may also help kill cancer cells. Dietary supplements (curcumin, vitamin D, vitamin K2, vitamin K1, B-6, high selenium broccoli sprouts, epigallocatechin gallate, L-carnitine, garlic extract, genistein, zinc amino chelate, mixed toxopherols, ascorbic acid, D-limonene) can block different targets in the cancer cell simultaneously and may slow down cancer growth. Giving gemcitabine hydrochloride, paclitaxel albumin-stabilized nanoparticle formulation, and metformin hydrochloride with a dietary supplement may work better in treating patients with metastatic pancreatic cancer.*
Status: Completed

45. NCT02404428. Utilizing MRI to Study the Effect of Sulforaphane on Prostate Cancer. [R. Mithen, Institute of Food Research, U.K.]. *Prostate cancer is a major public health problem and there is a strong need of new preventive strategies based on drug and lifestyle interventions. It is now well-established that healthy eating patterns and increasing physical activity can prevent or delay prostate cancer progression. Intake of cruciferous vegetables (e.g. broccoli, cabbage, cauliflower, Brussels sprouts, kale) has been associated with decreased risk of prostate cancer progression; however the underlying biological mechanisms remain unknown. The investigators propose to undertake a pilot study on a group of men with early prostate cancer on active surveillance to determine whether a diet rich in broccoli will induce changes in tumor size and blood flow measured by conventional Magnetic Resonance Imaging (MRI) techniques. Men with early prostate cancer on active surveillance who have visible cancer lesions on MRI will be recruited onto this double-blinded randomized intervention and they will be asked to eat one portion of broccoli soup per week for 6 months. The investigators will test two varieties of broccoli(standard and 'Beneforte extra' broccoli) that are able to deliver two different levels of sulforaphane (SF), an active compound extensively studied for its potential anticancer properties. This study will involve MRI scans, blood and urine collection before and after a 6 month intervention period. This study design will not only allow us to observe diet-induced changes within the prostate but also at the systemic level. In addition, participant's lifestyle (habitual diet and physical activity) will be assessed by food diaries and exercise questionnaires.*
Status: Enrolling by Invitation

46. NCT02561481. Sulforaphane Treatment of Children With Autism Spectrum Disorder (ASD). [A. W. Zimmerman, University of Massachusetts, Worcester]. *ASD is a diverse disorder starting in early childhood and characterized by social communication impairment as well as restricted interests and repetitive behaviors. It affects 1:68 children and is an enormous medical and economic problem for which there is no established, mechanism-based treatment. Sulforaphane is an isothiocyanate derived from broccoli, and has potent activity in transcriptionally up-regulating genes that control mechanisms whereby aerobic cells protect themselves against oxidative stress, mitochondrial dysfunction, and inflammation.*
This study is a clinical trial of oral sulforaphane (as broccoli seed powder) in 50 boys and girls (3-12 years) with ASD in 3 phases over 36 weeks. In Phase 1, 25 children will receive active drug and 25 will receive placebo for 15 weeks; in Phase 2, all children will receive sulforaphane from 15-30 weeks; in Phase 3, children will receive no treatment for 6 weeks. Study visits will take place at screening, 7, 15, 22, 30 and 36 weeks, when the Ohio Autism Clinical Clinical Impressions Scale - Severity and Improvement (OACIS-S and OACIS-I), Aberrant Behavior Checklist (ABC) and Social Responsiveness Scale (SRS) will be recorded. Children will be monitored with physical examinations and for toxicity with clinical laboratory studies and examine possible biomarkers: Nuclear factor-erythroid factor 2 (Nrf2), oxidative stress and mitochondrial function, the mechanistic target of rapamycin (mTOR) pathway and cytokine expression. In addition, prior to the main clinical trial, a pilot study will be carried out in 10 children with ASD, 6-12 years of age, who will receive sulforaphane, 2.2 micromoles/kg daily for 14 days. Blood and urine samples before and at the end of treatment will be collected, in order to measure several parameters that are likely to demonstrate expected effects of sulforaphane, to standardize the assays and procedures, and to determine the most effective measures.
Status: Recruiting

47. NCT02592954. Effect of Broccoli Sprout Extract on Keratinocyte Differentiation in Normal Skin. [B. Cohen, Johns Hopkins University]. *Adult participants will apply a broccoli sprout extract-jojoba oil compound to one arm every night under occlusion for 1 week. Jojoba oil alone will be applied to the other arm. At the end of 1 week, a 6mm punch biopsy will be taken from both arms and analyzed via polymerase chain reaction (PCR) and immunohistochemistry for differences in various skin proteins.*
Status: Recruiting

48. NCT02614742. SFX01 After Subarachnoid Haemorrhage. [D. Bulters, Evgen Pharma, London, United Kingdom]. *This is a Safety, Tolerability, Pharmacokinetic and Pharmacodynamic Study of SFX-01 in Subarachnoid Haemorrhage, with exploratory evaluations of efficacy.*
Status: Not Yet Recruiting

49. NCT02654743. Open Label of Clinical Trial of Sulforaphane in Children With Autism. [R. Hendren, University of California, San Francisco]. *This is an open-label, 4-month study examining the effects of Sulforaphane (SF) on behavior in children with ASD and the correlation between behavior change and urinary metabolites. The goal is to determine a potential mechanism of action of SF in this population.*
Status: Enrolling by Invitation

50. NCT02656420. Broccoli Sprout Dose Response. [T. Kensler, Johns Hopkins Bloomberg School of Public Health]. *This study will examine the extent to which lower doses of a broccoli-derived beverage enhance the detoxication of air pollutants excreted in urine as compared to an maximal dose shown to be effective previously.*
Status: Enrolling by Invitation

51. NCT02677051. Sulforaphane in a New Jersey (NJ) Population of Individuals With Autism. [W. G. Johnson, Rutgers, The State University of New Jersey]. *This study is a double blind treatment trial that will test if sulforaphane improves core symptoms in autism. The investigators expect to see clinical improvement in some of these areas. Sulforaphanes come from eating certain vegetables such as broccoli. The investigators will be using a preparation that gives specific and reproducible amounts. The investigators will also test specific chemicals and genes needed for sulforaphane usage to try to understand differences in response.*
Status: Not Yet Recruiting