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PROJECT TITLE: Sulforaphane treatment of children with Autism Spectrum Disorder

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14. ABSTRACT

This randomized clinical trial seeks to investigate the effect of sulforaphane, an isothiocyanate obtained from 3-day-old broccoli sprouts, on children with autism spectrum disorder (ASD). Sulforaphane has several possible modes of action that may benefit ASD through common cellular mechanisms that underlie its heterogeneous phenotypes. The three specific aims of the study are: (1) to determine if there are measurable effects on social responsiveness and problem behaviors during treatment with orally administered sulforaphane in 3-12-year old boys and girls with ASD; (2) to determine if treatment with sulforaphane is safe and well tolerated; and (3) to elucidate cellular biomarkers that support the hypothesized mechanism of action of sulforaphane in ASD. The study design consists of a short Pilot trial, to identify specific biomarkers for further study, and the Main clinical trial, with a double-blind, placebo-controlled, phase-2 crossover design. Outcome measures include analyses of blood and urine samples as well as scores on clinician- and parent-completed behavioral assessments. Analyses and assessments will be done at several specific points over the course of the study. To-date, the Pilot trial of 10 children has been completed, and all study participants for the Main trial have completed their visits. The plan for the next performance period is to complete the data entry into the electronic database, finish performing the biomarker analysis, perform statistical analysis of the study data, and author and publish the study manuscript.

15. SUBJECT TERMS
Sulforaphane, Autism Spectrum Disorder (ASD), Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS), Ohio Autism Clinical Impressions Scale (OACIS)

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Section 1: Introduction

This project seeks to investigate the effect of sulforaphane on children with autism spectrum disorder (ASD). Sulforaphane, an isothiocyanate obtained from 3-day-old broccoli sprouts, has several possible modes of action that may benefit ASD through common cellular mechanisms that underlie its heterogeneous phenotypes. Sulforaphane crosses the blood brain barrier and is bioavailable orally. The study will enroll 50 children with moderate to severe autism, between 3 and 12 years old, in a randomized, double-blind, placebo-controlled phase-2 clinical trial with a crossover design. At several specific points over the course of the study, clinicians will complete the Ohio Autism Clinical Impressions Scale (OACIS) and collect blood and urine samples from each child. Parents will also complete the Aberrant Behavior Checklist (ABC) and the Social Responsiveness Scale (SRS) at these points. Comparing the data from each of these assessments and analyzing the collected samples will provide information on both the behavioral and cellular effects of sulforaphane.

Section 2: Keywords

Sulforaphane, Autism Spectrum Disorder (ASD), Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS), Ohio Autism Clinical Impressions Scale (OACIS)

Section 3: Accomplishments

***** All accomplishments detail the cumulative performance of the study till date *****

What are the major goals of the study?

As per the SOW, following are the major specific aims of the study:

SPECIFIC AIMS 1 AND 2: Clinical trial: To determine if there are measurable effects on social responsiveness and problem behaviors during treatment with orally administered Sulforaphane-rich Broccoli Seed Powder (referred to as sulforaphane hereafter) in 3-12 years old boys and girls with ASD; To determine if treatment with sulforaphane is safe and well tolerated.

SPECIFIC AIM 3: To elucidate cellular biomarkers that support the hypothesized mechanism of action of sulforaphane in ASD. Blood and urine samples will be collected at UMass in Pilot and Main clinical trial, processed, stored and shipped on dry ice to Johns Hopkins.

What was accomplished under these goals?

In the last three years since receiving the grant, we completed the Pilot study and sent the collected blood and urine samples to Johns Hopkins for analysis, and conducted a preliminary analysis of the biomarkers pertinent to this study (SPECIFIC AIM 3). We have concluded the study visits of the Main clinical trial (SPECIFIC AIMS 1, 2, and 3). These accomplishments are described in more detail below.

During the first year of the study, we completed the study staff requirement of the Clinical Research Assistant and the Primary Care MD. We also organized training sessions to assure ratings reliability on the Ohio Autism Clinical Impressions Scale (OACIS), and to review consent/assent procedures and patient privacy protections. We were unable to move forward with the human subjects aspect of the research because of pending DoD HRPO approval. The only change we were asked to make by the HRPO after 3 months of waiting was to change our research monitor. Once we received the DoD HRPO approval on 12/21/2015, we started recruitment activities. Once recruitment was underway, however, enrollment proceeded at the anticipated rate. We successfully met our target sample size of 10 participants for the Pilot study over the course of January, February, and March 2016.

Participants for the Pilot study were screened over the phone to verify that they met the preliminary eligibility criteria. They then came to the study clinic for the screening appointment and for the follow-up appointment two weeks later. For the two weeks between screening and follow-up appointments, participants took a daily dose of sulforaphane prescribed based on their weight. At each appointment, blood and urine samples were collected, processed, stored, and shipped on dry ice to Johns Hopkins. A preliminary analysis of these samples was done to help elucidate cellular biomarkers for the Main clinical trial. These results were reported in a previous quarterly report, and are also reproduced below for reference purposes.

While conducting the Pilot study, we also continued recruitment for the Main clinical trial. After completion of the Pilot (final follow-up took place on 04/01/2016), we began scheduling screening appointments for the main clinical trial. At the end of the first year we had 9

participants actively enrolled in the Main trial. During the second year, we continued enrolling participants, and at the time of second annual report, we had 16 participants actively enrolled in the study, 20 had completed the study procedures, and 10 had completed phone screening and were pending enrollment for the screening visit in the upcoming several weeks. During the third year, we continued study participant enrollment, and as of writing this report, we have completed all study participant visits. Study enrollment report appears below:

Data as of: July 30, 2018

Recruitment Start Date: Pilot study: 11 January 2016, Main study: May 04, 2016

Target N: pilot study: 10, main study: 50

Randomized N: Pilot study: 0 (open label study, so no randomization), main study: 48

Active N: Pilot study: 0, main study: 8

Completed N: Pilot study: 10, main study: 48

Dropouts:

Dropouts (cumulative)	Reason for Dropout
5	Five participants dropped out due to refusal to take study drug; of these, two dropped out after completing the double-blind phase of the study, so the data from these two participants will be used for analysis.
1	One participant dropped out due to lack of improvement and an inability to maintain the time commitment required by the study.
2	Two participants dropped out due to experiencing sleep disturbances while taking the study drug.
1	One participant dropped out due to experiencing diarrhea while taking the study drug; this participant also struggled with the taste of the study drug.
1	One participant dropped out due to lack of improvement and continuing issues with preexisting gastrointestinal reflux; however, this participant dropped out after completing the double-blind phase of the study, so the data from this participant will be used for analysis.
1	One participant dropped out after refusing to take the study drug for several weeks and subsequently experiencing a possible seizure (while off of the study drug). This participant dropped out after completing the double-blind phase of the study, so the data from this participant will be used for analysis.
1	One participant dropped out due to lack of improvement in the study and continuing irritability. This participant dropped out after completing the double-blind phase of the study, so the data from this participant will be used for analysis.

1	One participant dropped out due to family's inability to maintain the time commitment required by the study while dealing with ongoing external factors (allergies, illness). This participant dropped out after completing the double-blind phase of the study, so the data from this participant will be used for analysis.
1	One participant dropped out due to difficulty taking the study drug, as well as ongoing gastrointestinal issues (experienced vomiting and diarrhea both while taking the study drug and during periods when study drug had been discontinued).

Lost to follow-up: 0

Enrollment in the Main clinical trial lasts 36 weeks and involves 6 visits to the study clinic (at screening, 7 weeks, 15 weeks, 22 weeks, 30 weeks, and 36 weeks). At each visit, study personnel do a physical exam of the child and collect blood and urine samples. These samples are processed and analyzed both for safety monitoring and for cellular biomarker elucidation. The clinician also completes the Ohio Autism Clinical Impressions Scale – Severity (OACIS-S) at screening and Improvement (OACIS-I) at all subsequent visits. Parents are asked to complete the Aberrant Behavior Checklist (ABC) and the Social Responsiveness Scale (SRS) at each study visit. Changes in scores on these assessments will serve as outcome measures for the study. Some additional assessments are performed only at the screening visit: the Autism Diagnostic Observation Schedule (ADOS-2) is used to confirm the ASD diagnosis, and the Vineland and Leiter-3 are used to provide a more complete understanding of the child's level of functioning.

There are three phases of participation in the study. In Phase 1 (15 weeks), half of participants receive sulforaphane and half of participants receive placebo. This phase is double-blinded; the pharmacy randomizes participants in either the sulforaphane or placebo group. In Phase 2 (15 weeks), all participants receive sulforaphane, regardless of what group they were in before. During Phases 1 and 2, participants take a daily dose of the dispensed study medication, calculated based on their weight. In Phase 3 (6 weeks), all participants discontinue sulforaphane.

By comparing data for each participant from the scheduled time points during the different phases of the study, we will be able to determine the effects of sulforaphane and elucidate the mechanisms behind any observed changes in behavior.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Preliminary report of progress in the study was presented at American Academy of Neurology Annual Meeting in Los Angeles in April 2018.

What do you plan to do during the next reporting period to accomplish the goals?

We started enrollment in our study 6 months late due to delay obtaining regulatory approvals. All study visits were completed by June 2018. Now that we have completed our study participant visits, we are finishing up the database entry and our plan for the next reporting period is to continue finishing the biomarker analysis, perform the statistical analysis on the study data and proceed with drafting and publishing the manuscript.

Section 4: Impact

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

The clinical trial has raised considerable interest in the autism community with respect to sulforaphane and other natural compounds that may have therapeutic benefits.

Preliminary findings:

N/A

Section 5: Changes/Problems

Changes in approach and reasons for change

N/A

Actual or anticipated problems or delays and actions or plans to resolve them

Recruitment and enrollment activities were significantly delayed while DoD HRPO approval was pending. Because of this delay, our enrollment of study subjects began several months later than anticipated and continued to lag the original enrollment targets. Due to this, we finished the study enrollment almost 6 months late – by end of June 2018.

Changes that had significant impact on expenditures

N/A

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Section 6: Products

Publications, conference papers, and presentations

Preliminary report of progress in the study was presented at American Academy of Neurology Annual Meeting in Los Angeles in April 2018.

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other products

Nothing to report

Section 7: Participating & Other Collaborating Organizations

What individuals have worked on the project?

Name:	Andrew Zimmerman, MD
Project Role:	Principal Investigator (PI)
Researcher Identifier:	None
Contribution to Project:	Dr. Zimmerman supervises recruitment, enrollment, study implementation, monitoring of side effects, data management and ensures that the research is conducted in line with the ethical provisions of the University of Massachusetts Medical School. He oversees and assures accurate data collection and analysis. He will present data at a national meeting, and prepare manuscripts for publication in peer-reviewed journals.

Name:	Kanwaljit Singh, MD MPH
Project Role:	Study Coordinator/Instructor in Pediatrics
Researcher Identifier:	None
Contribution to Project:	Dr. Singh assists the PI in the planning and implementation of all aspects of the study. He oversees the day to day operation of the study, including recruitment, screening procedures, scheduling, coordinating physical examinations, outcome measures and administration of medication. He works with our collaborators at Johns Hopkins to supply the Research Pharmacy with drug and placebos, and ensure timely delivery of medication to the participants. He assists with phlebotomies, blood sample preparation and shipments to the Cullman Chemoprotection Laboratory at Johns Hopkins. He is also responsible for data collection and storage in accordance with FDA guidelines, and oversees the collection of safety data and adverse event reporting for the Data Safety Monitoring Board (DSMB). We are requesting permission for Dr. Singh to carry out statistical analysis of the study data.

Name:	Susan Connors, MD
Project Role:	Primary Care MD
Researcher Identifier:	None
Contribution to Project:	Dr. Connors assists the PI in supervising and assisting with all medical aspects of the study, including review of medical

	histories during recruitment and screening, examinations of participants at each visit, and reporting of all potential side effects of treatment. She reviews participants' pre- and postnatal histories and development, current and past medications and allergies; records clinical data; and responds to parents' calls or emails with respect to medical questions or concerns related to the study. If necessary, she communicates with participants' pediatricians regarding questions about and concerns during the study, e.g., intercurrent illnesses, and reports any concerns to the PI and DSMB.

Name:	Ann Foley, EdM
Project Role:	Psychologist
Researcher Identifier:	None
Contribution to Project:	Ann Foley performs ADOS, Vineland, and Leiter-3 assessments.

Name:	Louise Maranda, PhD
Project Role:	Biostatistician
Researcher Identifier:	None
Contribution to Project:	Dr. Maranda has left UMass and her duties will be assumed by Dr. Kanwaljit Singh.

Name:	Eileen Diggins
Project Role:	Clinical Research Assistant
Researcher Identifier:	None
Contribution to Project:	Eileen Diggins assisted in recruitment and scheduling of appointments for screening, reporting and recording data from baseline assessments and outcome measures. She worked with other members of the team to communicate effectively with families in a timely manner, in order to assure both safety and compliance with the drug regimen and collection of data. She reported any concerns of families to the PI, Research Coordinator and Primary Care MD.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Louise Maranda, study statistician, is no longer available for consultation or data analysis. After our study enrollment finished, Eileen Diggins recently left UMass and will be starting a graduate program in Psychology later this fall.

What other organizations were involved as partners?

Johns Hopkins University School of Medicine, Baltimore, MD

Collaborator: This is the site where biomarker assays are being performed. This site will perform under a sub-contract. No study participant enrollment will take place at this site. They have obtained their own IRB approval.

Sub-contract Investigators.: Paul Talalay, MD and Jed Fahey, PhD

Co-investigator: Hua Liu, PhD

Section 8: Special Reporting Requirements:

None

Section 9: Appendices

None