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Award Number: W81XWH-15-1-0156

TITLE: Sulforaphane Treatment of Children with Autism Spectrum Disorder

PRINCIPAL INVESTIGATOR: Andrew Zimmerman

CONTRACTING ORGANIZATION: UNIVERSITY OF MASSACHUSETTS
Worcester, MA 01655

REPORT DATE: July 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2016	ANNUAL	1 Jul 2015 - 30 Jun 2016

4. TITLE AND SUBTITLE
Sulforaphane treatment of children with Autism Spectrum Disorder

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0156

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

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 12 out of the target 50 children for the Main trial have been enrolled. Our plan for the next reporting period is to continue recruiting and enrolling participants in the Main clinical trial.

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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	18	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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Section 1: Introduction

This project seeks to investigate the effects 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

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 year- 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 mechanisms of action of sulforaphane in ASD. Blood and urine samples from the Pilot and Main clinical trials are assayed for biomarkers by Co-investigators at Johns Hopkins.

What was accomplished under these goals?

In the year since receiving the grant, we have completed the Pilot study and sent the collected blood and urine samples to Johns Hopkins for analysis (SPECIFIC AIM 3). We have also begun conducting the Main clinical trial, and are continuing the process of recruitment, enrollment, and data collection (SPECIFIC AIMS 1, 2, and 3). These accomplishments are described in more detail below.

During the first quarter of this reporting period, we completed the study staff by recruiting the Clinical Research Assistant (Eileen Diggins, BA) and the Primary Care MD (Susan Connors, 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 recruitment of human subjects for the research, pending DoD HRPO approval. We were asked by the HRPO to change our research monitor, then received DoD HRPO approval on 12/21/2015 and started recruitment activities.

We had initially anticipated being able to enroll the 10 participants for the Pilot study within the first quarter of this reporting period. The delay in receiving DoD HRPO approval necessitated pushing back our quarterly target enrollments. 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 the prescribed daily dose of sulforaphane based on their weight. At each appointment, blood and urine samples were collected, processed, stored, and shipped on dry ice to Johns Hopkins. Analysis of these samples will help elucidate cellular biomarkers for the Main clinical trial.

While conducting the Pilot study, we also continued recruitment for the Main clinical trial. In January 2016, we sent flyers and a cover letter to the parents of children formerly seen in clinic

by Dr. Zimmerman (PI) who might qualify for the study. A description of the study was also sent out in an electronic newsletter. All recruitment materials contained contact information for study personnel (phone and email), and were approved by the IRB prior to sending. Families who wished to receive more information contacted study personnel. For those who were interested in moving forward with the study, we went through the phone screen and added their names to a list of people to be contacted when the Main clinical trial began. After completion of the Pilot (final follow-up took place on 04/01/2016), we began scheduling screening appointments for children on this list.

To date, we have phone-screened 44 potential participants for the Main clinical trial. Of those, 12 are currently enrolled as active participants, and 12 are pending enrollment. One participant had abnormal laboratory studies at the screening visit and will require further pediatric assessment prior to participating in the study. The remaining 20 are inactive, including 1 participant who elected to drop out of the study after being enrolled for 7 weeks. A breakdown of the phone screen outcomes so far can be found in *Table 1* below. Our target enrollments for the Main clinical trial were 7 participants during Quarter 2, 10 participants during Quarter 3, and 8 participants during Quarter 4. However, due to the delayed DoD HRPO approval described above, Main clinical trial enrollment only started four months prior to the submission of this report. Given this, our current enrollment rate is on target.

44 total phone screens		24 active		12 enrolled		
				12 pending enrollment		3 scheduled screening appointments
						9 pending scheduling
		20 inactive		1 drop out		
				10 ineligible		2 ineligible at screening appointment
						8 ineligible based on phone screen
				9 uninterested		2 presumed (no-show at screening appointment)
						7 stated (time travel, blood draws, etc.)

Table 1. Breakdown of phone screen outcomes for Main clinical trial, to date.

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 (clinical laboratory tests) 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 to 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 and return at 36 weeks for follow up.

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.

We have processed the pre- and post-treatment blood samples from all 10 patients in the pilot study to optimize the protocol for collecting, processing and storage of the samples. Expression levels of 17 biomarkers related to our hypothesized mechanisms of the action of sulforaphane have been analyzed in these samples using both isolated blood cells (peripheral blood mononuclear cells or PBMCs) and plasma. By comparing the differences between pre- and post-treatment blood samples for each individual in the pilot study, we have selected a panel of biomarkers which showed sensitivity and consistent responsiveness to sulforaphane treatment. We will focus initially on this panel of biomarkers in the Main clinical trial, and we will continue to evaluate other markers with remnant biological samples from the Pilot study.

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

In order to facilitate screening, the Clinical Research Assistant (Eileen Diggins) attended a training conference for the Autism Diagnostic Observation Schedule (ADOS-2), the assessment used to confirm participants' ASD diagnosis. Once she has attained inter-rater reliability, she will be available as an alternate study team member who can administer this assessment.

How were the results disseminated to communities of interest?

Nothing to report

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

Our plan for the next reporting period is to continue recruiting and enrolling participants in the Main clinical trial. During the upcoming quarter, we aim to enroll one or two participants per week. This will bring us to about one-half of our target enrollment of 50 by the end of the next quarter. By the time of the next annual report, we anticipate that we will have enrolled our target 50 participants. Our goal is to enroll the final wave of participants by the end of May 2017. These final participants would be finishing their participation by the beginning of February 2018, allowing us the final six months of the allotted study time to analyze and synthesize the collected data.

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?

We have received positive feedback from families of children with ASD regarding our conduct of the study, which has enhanced our recruiting efforts.

Section 5: Changes/Problems

Changes in approach and reasons for change

Nothing to report

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

Recruitment and enrollment activities were delayed while DoD HRPO approval was pending. Because of this delay, our enrollment of study subjects began several months later than anticipated. In order to ensure that we are still able to meet our target of 50 participants for the study, we plan to enroll more participants per quarter than initially projected. Our original plan was to enroll half of the participants within the first year, and gradually taper off enrollment in the following years (moving from 7 or 8 participants per quarter to 3 or 2). We have adjusted this projection, and now intend to continue enrolling 7 or 8 participants per quarter through the second year of the study. This should bring us back on track for reaching our target sample size within the allotted time.

Changes that had significant impact on expenditures

We had unanticipated charges for manufacturing placebos for the study ($11,040.72, paid to Stephen Hoag, PhD, University of Maryland), which required reallocation of budget items within the grant.

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

On May 20, 2016, we presented the preliminary findings (from our previous trial in adult males with ASD) and a description of our current project at the 6th Annual Research Retreat hosted by the UMass Center for Clinical and Translational Science. The Retreat consisted of a poster session with over 100 participants from across the 5 UMass campuses, as well as several mini symposia and oral presentations.

Website(s) or other Internet site(s)

Our study is listed on ClinicalTrials.gov, an online database affiliated with the U.S. National Institutes of Health: https://clinicaltrials.gov/ct2/show/NCT02561481.

Technologies or techniques

We are planning additional laboratory studies of mitochondrial functions in collaboration with Elisabet Mandon, PhD at UMass.

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
Nearest person month worked:	1
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 of Pediatrics
Researcher Identifier:	None
Nearest person month worked:	12
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).

Name:	Susan Connors, MD
Project Role:	Primary Care MD
Researcher Identifier:	None

Nearest person month worked:	4
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
Nearest person month worked:	1
Contribution to Project:	Ann Foley performs ADOS, Vineland, and Leiter-3 assessments.

Name:	Louise Maranda, PhD
Project Role:	Biostatistician
Researcher Identifier:	None
Nearest person month worked:	1
Contribution to Project:	Dr. Maranda will conduct this project's statistical analyses. She assists in the planning for the clinical trial design, data handling and storage, and evaluation of outcomes.

Name:	Eileen Diggins
Project Role:	Clinical Research Assistant
Researcher Identifier:	None
Nearest person month worked:	9
Contribution to Project:	Eileen Diggins assists in recruitment and scheduling of appointments for screening, reporting and recording data from baseline assessments and outcome measures. She works 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 reports 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?

Nothing to report

What other organizations were involved as partners?

Johns Hopkins University School of Medicine, Baltimore, MD

Collaborator: This is the site where biomarker assays will be performed. This site is performing under a sub-contract. No study participant enrollment will take place at this site. They have obtained approval for their involvement in the studies (Pilot and Main) from the Johns Hopkins IRB (#IRB00084331).

Sub-contract Investigator.: Jed W. Fahey, Sc.D.

Co-Investigator: Hua Liu, PhD

Study Staff: Kristina L. Wade, M.S.

Section 8: Special Reporting Requirements:

None

Section 9: Appendices

Appendix 1: UMass IRB Annual Continuing Review Approval letter (on page 16)

University of Massachusetts
UMASS Medical School

6/27/2016

Andrew Zimmerman, MD
University of Massachusetts
Pediatrics

Dear Dr. Zimmerman:

The IRB reviewed the following:

Type of Submission:	Continuing Review
Review Type:	Committee
Project Title:	Sulforaphane Treatment of Children with Autism Spectrum Disorder (ASD).
Investigator:	Andrew Zimmerman, MD
IRB ID:	H00007832_4
Funding Agency:	U.S. DEPARTMENT OF DEFENSE
Grant Title:	Sulforaphane Treatment of Children with Autism Spectrum Disorder (ASD).
Grant ID:	AR140087
IND or IDE:	Name: Sulforaphane, IND #: 127062
IRB Review Date:	6/21/2016
Documents Reviewed:	SFN Study Protocol AME2 CLEAN 120415.docx H00007832_ Research Monitor v2 111815.pdf SFN Study Demographics CLEAN 060215.docx SFN Study Adverse Event Log 041415.docx i127062 Study May Proceed (COR-INDAD-01)signed.pdf SFN Study Grant Application SFN Pilot Study Advertisement CLEAN 111815.doc SFN Study Medical History 2 041415.docx SFN Study Screening Visit Checklist CLEAN 060215.docx H00007832 Investigator Brochure 081815.docx SFN Study Physical Exam 041415.docx SFN Study Adverse Event Codes 081815.docx SFN Pilot Study Consent CLEAN 081815.docx SFN Study, Newsletter Blurb, 11.13.2015.docx SFN Study Assent CLEAN 060215.docx SFN Study Phone Screen CLEAN 060215.docx SFN Study Sample Collection Log 041415.doc SFN Study Flyer CLEAN 111815.doc

	SFN Study Physician Letter CLEAN 060215.docx SFN Pilot Study Assent CLEAN 060215.docx SFN Study Inclusion Exclusion Criteria CLEAN 060215.docx SFN Study Advertisement CLEAN 111815.doc UMass Institutional Biosafety Committee approval SFN Study Medication Diary 041415.doc SFN Study Study Diary Side Effects 041415.doc SFN Study Medical History 041415.docx SFN Pilot Study Recruitment Letter CLEAN 111815.docx SFN Pilot Study Flyer CLEAN 111815.doc Nutramax No FCOI letter 081815.pdf SFN Study Documentation of Assent Consent 041415.docSFN Study Visit Checklist CLEAN 060215.docx SFN Study Surveys.pdf SFN Study Medical History Parent Report CLEAN 060215.docx SFN Study HIPAA CLEAN 060215.docx SFN Study Recruitment Letter CLEAN 111815.docx SFN Study Consent CLEAN 081815.docx SFN Study Con Med Log 041415.doc H00007832 DSMB Report 1 052416.docx

The Committee determined that the research continues to present greater than minimal risk and the prospect of direct benefit to children (21 CFR 50.52). There are adequate provisions for soliciting assent (21 CFR 50.55) and the permission of the parents (21 CFR 50.55).

The IRB has reviewed the DoD grant listed above and found it to be consistent with research activities described in the protocol

The IRB approved the research from 7/21/2016 to 7/20/2017 inclusive. Before 6/5/2017 or within 30 days of closing the study, whichever is earlier, you are required to submit a completed Continuing Review Progress Report and necessary attachments to request continuing approval or study closure.

If continuing review approval is not granted before the expiration date of 7/20/2017, approval of this research expires on that date.

Stamped consent documents are included with this approval. Use these to document consent.

In conducting this research, you are required to follow the requirements listed in the INVESTIGATOR MANUAL.

Sincerely,

Sarah Saliba, CIP
Protocol Specialist, IRB

cc: Singh, Kanwaljit
Diggins, Eileen
Barron, Eyeisha, Human Subjects Protection Scientist, Human Research Protection Office, US Army Medical Research & Materiel Command (eyeisha.o.barron.ctr@mail.mil)