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 2017

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2017	ANNUAL	1Jul2016 - 30Jun2017

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)
Andrew W. Zimmerman, MD

E-Mail: Andrew.Zimemrman@umassmemorial.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of Massachusetts, Medical School
55 Lake Ave N
Worcester, MA 01655

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 36 out of the target 50 children for the Main trial have been enrolled. Our plan for the next reporting period is to finish recruiting and enrolling participants in the Main clinical trial and perform data analysis.

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	UU	15	19b. TELEPHONE NUMBER *(include area code)*

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

Table of Contents

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 two years since receiving the grant, we have 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 also been 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 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. Since that time we have continued enrolling participants, and as of writing this we have 16 participants actively enrolled in the study, 20 have completed the study procedures, and 10 have completed phone screening and are pending enrollment for the screening visit in the upcoming several weeks. The number of actively enrolled participants would have been higher, however a few participants dropped out of the study without completing a single follow-up visit, so we will have to replace these participants. A breakdown of the current enrollment screenshot so far can be found in *Table 1* below.

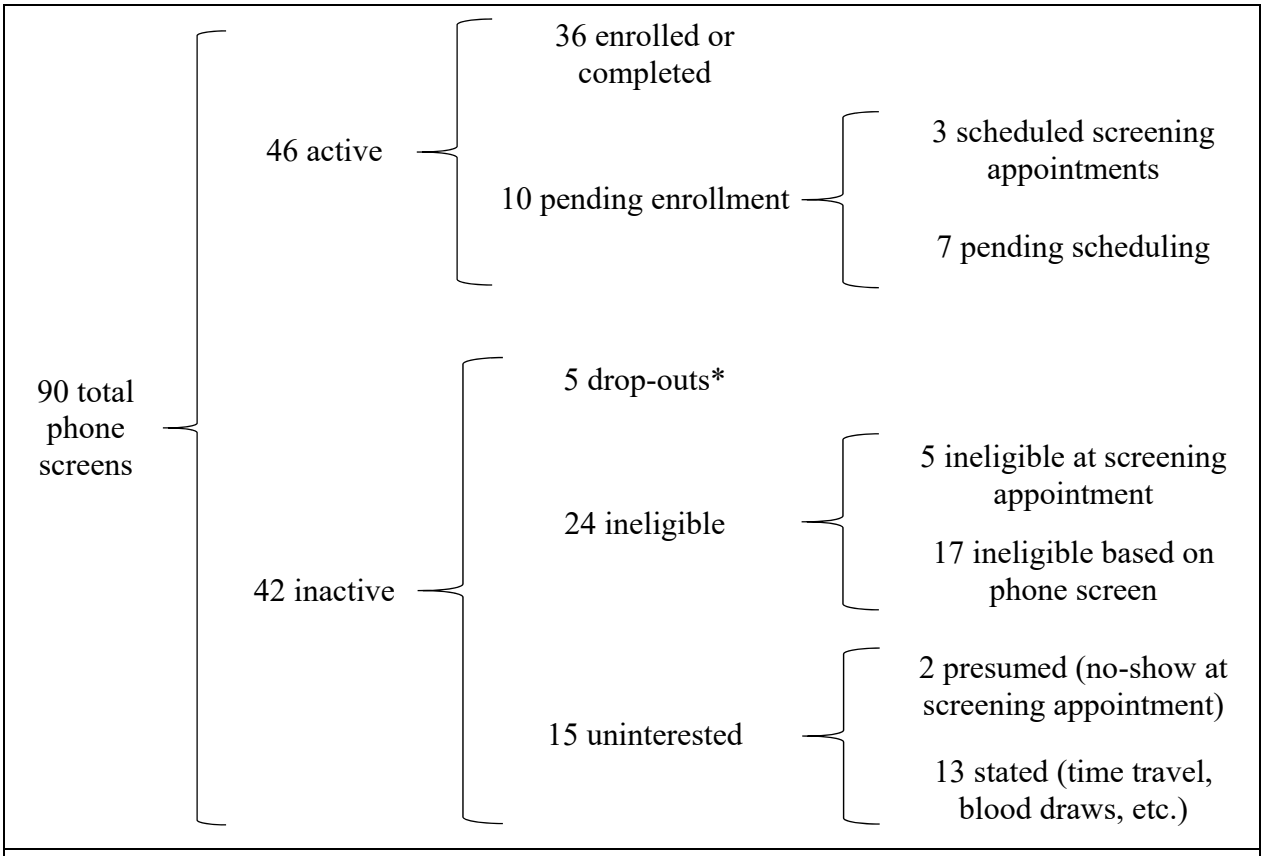


Table 1. Breakdown of phone screen outcomes for Main clinical trial, to date.

* Drop-outs: one participant dropped out due to participant's family unable to meet study visit commitments, two participants dropped out due to experiencing sleep difficulties, and two dropped out due to their refusal to take study drug (due to taste, smell etc.).

Two more participants dropped out after completing the double blind phase of the study (one because of continuing issues with preexisting GI reflux, and a second one dropped out due to refusal to take the study drug). However since both of these participants completed the double blind phase of the study, their study data will be usable for the final analysis.

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 Pediatric Grand Rounds at UMass on May 26, 2017.

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 completing recruiting and enrolling participants in the Main clinical trial. We have 10 participants pending enrollment which we hope to complete in the next few weeks. We are hopeful of completing the target enrollment of 50 participants in the Main clinical trial in the upcoming quarter. If we are able to finish the enrollment in the next few months, these final participants should be finishing their participation by the end of May 2018.

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:

1. **Urinary excretion of Dithiocarbamates (metabolic end product of Sulforaphane):** During the pilot study, we tried to collect 24 hour urine samples from the study participants at the second visit (follow-up visit after 2 weeks of study drug ingestion) in order to measure urinary excretion of dithiocarbamates (they are the metabolic end products of sulforaphane).

   Although we asked all participants to collect the 24 hour urine samples, in some cases it was difficult to acquire a complete 24 hour sample because some children were unable to cooperate, and because in some cases study participants took the study drug in the morning and then went to school for the next 8 hours (and thus missed the most important time period when much of the urinary excretion of sulforaphane metabolites occurs post ingestion).

   Nevertheless we were able to obtain full usable 24 hour samples from 5 participants, and the dithiocarbamate excretion was 49.9% ± 13% (range 37.2% - 68.4%). This is very close to a bioavailability of 50% that we anticipated from our study drug.

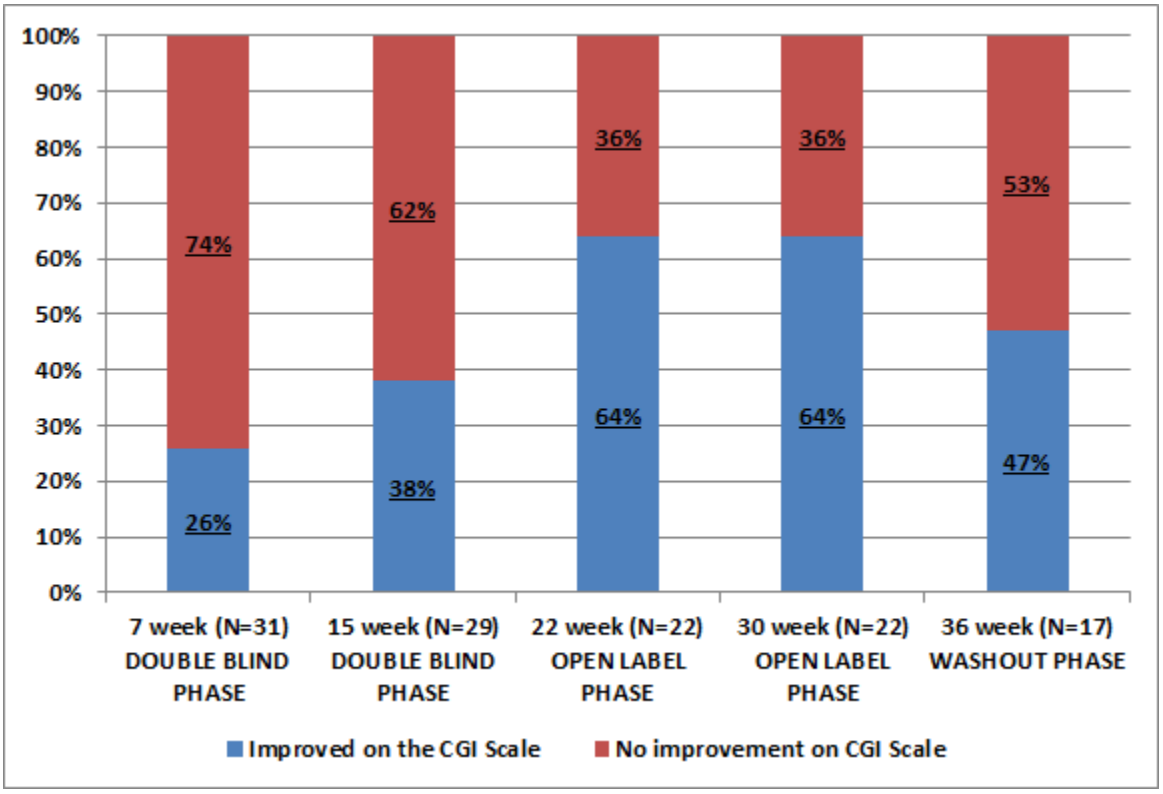
2. **Preliminary analysis of biomarkers and inflammatory cytokines before and after study drug intake in the pilot study:** As a part of the pilot study we also collected blood samples to do a preliminary analysis on a range of biomarkers, such as inflammatory cytokines, in order to get an idea about the trends of change in these markers after 2 weeks of sulforaphane administration. It took us some time to perfect the PBMC isolation protocol, and finally pre- and post-sulfpraphane administration data was available on 5 study partcicipants. The following biomarkers were studied:

   - **Heat shock protein 27 (Hsp27):** elevated in 3 out of 5 participants
   - **Heat shock protein 70 (Hsp70):** elevated in 3 out of 5 participants

- **Kelch-like ECH-associated protein 1 (KEAP1):** elevated in 3 out of 5 participants
- **Nuclear factor erythroid 2–related factor 2 (NRF2):** elevated in 2 out of 5 participants
- **Cyclooxygenase-2 (COX2):** decreased in 3 out of 5 participants
- **Interleukin-6 (IL6):** decreased in 4 out of 5 participants
- **Interleukin 1-beta (IL-1B):** decreased in 3 out of 5 participants
- **Inducible Nitric oxide synthase (iNOS):** decreased in 4 out of 5 participants
- **Tumor necrosis factor alpha (TNF-alpha):** decreased in 3 out of 5 participants

HSP27, HSP70, KEAP1, and NRF2 are the markers we expected sulforaphane to activate, and COX2, IL6, IL-1B, iNOS, and TNF-alpha are inflammatory markers that we expected will be inhibited by sulforaphane. It must be noted however that this was a preliminary analysis, on a small sample of study participants, who took sulforaphane for a relatively short period of time. Nevertheless, we are encouraged by the preliminary pilot study findings.

3. **Preliminary analysis of participants on the primary study outcome measure (Ohio Autism Clinical Global Impressions – Improvement scale):** We recently had an opportunity to perform a preliminary analysis to determine which participants in the Main trial were much or very much improved on the Ohio Autism Clinical Global Impressions – Improvement scale (the primary study outcome measure) as a part of a grand rounds presentation by the study PI, Dr. Andrew Zimmerman, at University of Massachusetts Medical School. A bar chart is included below describing the results.



Section 5: Changes/Problems

Changes in approach and reasons for change

Due to increased problems with maintenance of sleep in 2 children in the study (who were taking active drug), we revised the consent form to include this possible side effect, which was reviewed by the IRB.

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 has continued to lag the original enrollment targets. Still we are doing reasonably well with our enrollment so far and have 10 participants waiting enrollment in the next several weeks, bringing our total enrolled participant number to 46.

Changes that had significant impact on expenditures

Our study statistician (Louise Maranda) left UMass. We have requested permission from DOD to transfer her duties and allotted salary to Kanwaljit Singh, MD, MPH.

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

A progress report of the study was presented at Pediatric Grand Rounds at UMass on May 26, 2017.

Dr. Zimmerman and Dr. Fahey (our collaborator at Johns Hopkins) will be presenting on this subject at the International Society for Nutritional Psychiatry Research, Bethesda, MD, July 31, 2017.

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
Nearest person month worked:	
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
Nearest person month worked:	
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
Nearest person month worked:	
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:	
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:	
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
Nearest person month worked:	
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?

Dr. Louise Maranda, study statistician, is no longer available for consultation or data analysis.

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