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TITLE: Molecular Studies to Identify Mechanisms That Underlie Symptom Improvement in Microbiota Transfer Therapy Patients

PRINCIPAL INVESTIGATOR: Stephen J Walker

CONTRACTING ORGANIZATION: Wake Forest University Health Sciences

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Fort Detrick, Maryland 21702-5012

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4. TITLE AND SUBTITLE

Molecular Studies to Identify Mechanisms That Underlie Symptom Improvement in Microbiota Transfer Therapy Patients

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5b. GRANT NUMBER AR190127

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)

Wake Forest University Health Sciences
Medical Center Blvd
Winston Salem, NC 27157-0001

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Development 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

The purpose of this study is to perform molecular analysis (transcriptomic and metabolomic assays) on samples (whole blood, serum, and stool) derived from 84 adults with autism spectrum disorder (ASD) undergoing microbiota transfer therapy (MTT), and 84 adult controls, from an on-going CDMRP-funded clinical trial (James Adams, PI; Arizona State University) to identify mechanisms that underlie symptom improvement following MTT. Due to the onset of the coronavirus pandemic, recruitment at the ASU site has been severely curtailed since March 2020, and so sample collection and transfer to me has been limited to a portion of the initial 35 ASD sample sets, and 11 control sample sets. Because these samples need to be assayed in batches, although we have begun sample processing, we have not yet begun the molecular assays. We expect to get the assays underway in the Fall of this year (2022) and have requested, and been granted, a no-cost extension to complete the work in 2023.

15. SUBJECT TERMS

Autism spectrum disorder, gastrointestinal, microbiota transfer therapy, transcriptomics, microbiome, metabolomics

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
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Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. INTRODUCTION

The purpose of this study is to perform molecular analysis (transcriptomic and metabolomic assays) on samples (whole blood, serum, and stool) derived from 84 adults with autism spectrum disorder (ASD) undergoing microbiota transfer therapy (MTT), and 84 adults without ASD (controls), from an on-going CDMRP-funded clinical trial (James Adams, PI; Arizona State University) to identify mechanisms that underlie symptom improvement following MTT. In the subjects with ASD that receive MTT, whole blood, serum, and stool are collected at three time points: (1) baseline, (2) after 8 weeks, and (3) after 16 weeks, and assayed for gene expression and metabolite changes that occur over time. Profiles derived from whole blood, serum, and stool samples from individuals without ASD and who do not undergo MTT serve as 'normal' baseline transcriptomic and metabolomic profiles.

2. KEYWORDS

Autism spectrum disorder, gastrointestinal, microbiota transfer therapy, transcriptomics, microbiome, metabolomics

3. ACCOMPLISHEMENTS

- **What were the major goals of this project?**
  There are 7 tasks detailed on the SOW associated with this project. Task 1 requires procurement of the appropriate IRB and HRPO approvals. Tasks 2 and 3 describe sample processing, Tasks 4 and 5 describe acquisition of the molecular (transcriptomic and metabolomic) datasets; Task 6 describes metabolomic data analysis and visualization; Task 7 describes integrated omics data analysis.

- **What was accomplished under these goals?**
  To date, only Task 1: "Secure IRB and HPRO approvals to receive and use the specimens (human anatomical substances - HAS)" has been completed. As of March 2022, the sample sets (stool, plasma, and Paxgene whole blood) from 66 individuals (39 individuals receiving MTT and 27 NT controls) have been received in the PI's laboratory. Samples processing (total RNA from Paxgene blood; Task 2) has begun however molecular assays and data analyses described in Tasks 2-7 will not get underway until later in 2022 (November/December).

- **What opportunities for training and professional development has the project provided?**
  Nothing to report.

- **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?**
  Throughout the course of the next reporting period, we expect to receive most, if not all, of the remaining study samples. Throughout the period we will be processing the

samples on hand and, as sufficient numbers of samples have been prepared and qc'd for batch assay, we will initiate the molecular data generation and analyses.

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?**
  Nothing to report.

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**
  This project is entirely dependent on getting study samples (whole blood, serum, and stool) from Dr. Adams' clinical trial and therefore the delays Dr. Adams has experienced since March 2020 in being able to enroll and follow-up with participants in his study have impacted our ability to begin sample processing and data generation. Now that most of the relevant restrictions are easing, we anticipate that we can bring this project fully back online and begin receiving, processing, and assaying samples throughout the second reporting period (May 2021 through May 2022, and beyond into Y3). We have asked for and received a one year no-cost extension to allow for completion of the Tasks outlined in the SOW.

- **Changes that had a significant impact on expenditures**
  The inability to perform the lab work outlined in Tasks 2-7 over this last year has resulted in a significant reduction in expenditures during this reporting period. We anticipate that we will get back on track with expenditures in the second reporting period.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  Nothing to report.

6. PRODUCTS

- **Publications, conference papers, and presentations**
  Nothing to report.

- **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.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on this project?**
  - **Name:** Stephen Walker
  - **Project Role:** PI
  - **Researcher ID:** 0000-0002-0732-2366
  - **Nearest person month worked:** 2
  - **Contribution to project:** Dr. Walker is responsible for project oversight and management.
  - **Funding Support:** this award

  - **Name:** Trang Simon
  - **Project Role:** Technician IV
  - **Researcher ID:** n/a
  - **Nearest person month worked:** 2
  - **Contribution to project:** Ms. Simon is responsible for sample handling/processing.
  - **Funding Support:** this award

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  Yes (please see Other Support Document in Appendices).

- **What other organizations were involved as partners?**
  - **Organization Name:** Arizona State University
  - **Location of Organization:** Tempe Arizona
  - **Partner's contribution to the project:** Dr. James Adams provides samples (whole blood, serum, and stool) from participants in his CDMRP-funded clinical trial for this project.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

9. APPENDICES

Other Support Document.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

PHS OTHER SUPPORT
For All Application Types – DO NOT SUBMIT UNLESS REQUESTED

There is no "form page" for reporting Other Support. Information on Other Support should be provided in the format shown below.

*Name of Individual: Walker, Stephen J.
Commons ID: sjwalker

Other Support – Project/Proposal

ACTIVE

*Title: Prenatal Cell and Gene Therapy for Hemophilia A

*Major Goals: To prove the safety and efficacy of using cells as a FVIII delivery platform, and demonstrate the ability of IUTx to cure or improve HA phenotype, and defeat the immune-related hurdles that currently hinder clinical HA treatment.

*Status of Support: Active

Project Number: R01HL135853

Name of PD/PI: Almeida-Porada, G. /Porada, C.

*Source of Support: NHLBI

*Primary Place of Performance: Wake Forest University Health Sciences

Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/2017 – 06/2022

* Total Award Amount (including Indirect Costs):

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2018	0.36
2. 2019	0.36
3. 2020	0.36
4. 2021	0.36
5. 2022	0.36

*Title: Novel Microfluidic Biomarker Detection Platforms to Monitor In Vivo Effects of SPE and GCR Radiation, using Mice with Human Hematopoietic Systems

*Major Goals: Our goal is to identify early and persistent biomarkers of response that may identify susceptibility pathways, genes, and regulatory elements associated with onset and progression of human hematological disease (focusing on leukemia) following exposure to mission-relevant doses of space radiation.

*Status of Support: Active

Project Number: NASA 16-TRIRT_PROP-0072

Name of PD/PI: Almeida-Porada, G. /Porada, C.

*Source of Support: s/Baylor NASA Translational Research Institute for Space Health

*Primary Place of Performance: Wake Forest University Health Sciences

Name of Individual: Walker, Stephen J.
Commons ID: swalker

Project/Proposal Start and End Date: (MM/YYYY) (if available): 11/2017 – 07/2022

* Total Award Amount (including Indirect Costs):

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2018	1.20
2. 2019	1.20
3. 2020	1.20
4. 2021	1.20
5. 2022	1.20

*Title: Molecular Studies to Identify Mechanisms that Underlie Symptom Improvement in Microbiota Transfer Therapy Patients

*Major Goals: Aim #1: Evaluation of transcript expression changes that occur following full spectrum microbiota (FSB) treatment in individuals with ASD and chronic gastrointestinal symptoms. Aim #2: Evaluation of global metabolite changes that occur following FSB treatment in individuals with ASD and chronic gastrointestinal symptoms. Aim #3: Use a combined molecular profile (transcript expression and metabolite abundance) to derive mechanistic insights regarding symptom improvement following FSB treatment in individuals with ASD and chronic gastrointestinal symptoms.

*Status of Support: Active

Project Number: W81XWH2010275

Name of PD/PI: Walker, Stephen J.

*Source of Support: Department of Defense

*Primary Place of Performance: Wake Forest University Health Sciences

Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/2020 – 04/2022

* Total Award Amount (including Indirect Costs):

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2020	2.04
2. 2021	2.04
3. 2022	2.04

*Title: A Rapid and Simple Blood Test to Identify Gastrointestinal Inflammation in Children with ASD

*Major Goals: Characterize the gene expression profile of the ASDEC phenotype, including: (a) identification of a gene expression based molecular signature in peripheral blood, (b) validation of the blood-based biomarker that is sensitive and specific and can be useful as a minimally-invasive tool for guiding diagnosis and treatment of gastrointestinal inflammation in children with ASD and (c) testing the utility of the biomarker in pre- and post-treatment blood samples from a series of ASDEC patients.

Name of Individual: Walker, Stephen J.
Commons ID: swalker

*Status of Support: Active

Project Number: W81XWH2110294

Name of PD/PI: Walker, Stephen J.

*Source of Support: Department of Defense

*Primary Place of Performance: Wake Forest University Health Sciences

Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/2021 – 04/2024

* Total Award Amount (including Indirect Costs):

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	1.80
2. 2022	1.80
3. 2023	1.80
4. 2024	1.80

*Title: Molecular Characterization of a Large Cross-Sectional and Longitudinal Collection of Patients to Investigate Disease Progression in IC/BPS

*Major Goals: Objectives for this proposal are to use a sophisticated molecular profiling approach, in a broadly heterogeneous group of IC/BPS patients, to: (1) identify patient subgroups that share a similar disease etiology and are therefore likely to respond, as a group, to therapy that targets the underlying mechanism and, (2) identify gene expression modules that are predictive of disease progression in IC/BPS.

*Status of Support: Active

Project Number: R01 DK124599

Name of PD/PI: Walker, Stephen J.

*Source of Support: NIH/NIDDK

*Primary Place of Performance: Wake Forest University Health Sciences

Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/2020 – 04/2023

* Total Award Amount (including Indirect Costs):

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2020	1.80
2. 2021	1.80
3. 2022	1.80
4. 2023	1.80

*Title: Assessment of Urinary Polymerase Chain Reaction (PCR) and Next Generation Sequencing (NGS) Technology in the Evaluation and Management of Females with Chronic Bladder Pain and Cystitis-Like Symptoms. Protocol 20211649

Name of Individual: Walker, Stephen J.
Commons ID: swalker

*Major Goals: To perform a prospective clinical evaluation of the risks and benefits of PCR/NGS technology in the practical clinical evaluation and management of patients with bladder pain syndrome (BPS) and chronic cystitis-like symptoms (CCS) pursuant to the protocol.

*Status of Support: Active

Project Number: N/A

Name of PD/PI: Walker, Stephen J.

*Source of Support: MicroGenDx

*Primary Place of Performance: Wake Forest University Health Sciences

Project/Proposal Start and End Date: (MM/YYYY) (if available): 10/2021 – 10/2026

* Total Award Amount (including Indirect Costs):

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	0.06
2. 2023	0.06
3. 2024	0.06
4. 2025	0.06
5. 2026	0.06

*Title: Double-Blind, Placebo Controlled Study to Establish the Safety and Efficacy of Super-Concentrated, Freeze-Dried Aloe Vera in the Management of the Symptoms of Interstitial Cystitis

*Major Goals: The primary objective of this clinical trial is to monitor the safety and efficacy of Desert Harvest's super-concentrated, freeze-dried aloe vera in the management of the symptoms of interstitial cystitis. The symptoms that will be monitored will include urinary frequency, nocturia, dysuria, urinary urgency, and suprapubic pain. Response to therapy will be monitored through questionnaires including the Quality-of-Life Assessment, IC Symptom/Problem Index, MOS Sexual Functioning Guide, University of Wisconsin Symptom Survey, Health Status Questionnaire, Genitourinary Pain Index, and 24-Hour Voiding Diaries.

*Status of Support: Active

Project Number: N/A

Name of PD/PI: Walker, Stephen J.

*Source of Support: Desert Harvest, Inc.

*Primary Place of Performance: Wake Forest University Health Sciences

Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2021 – 03/2023

* Total Award Amount (including Indirect Costs):

* Person Months (Calendar/Academic/Summer) per budget period.

Name of Individual: Walker, Stephen J.
Commons ID: swalker

Year (YYYY)	Person Months (##.##)
1. 2021	1.20
2. 2022	1.20
3. 2023	1.20

*Title: Lung Organ Tissue Equivalent Platform for Modeling Chlorine Gas Toxicology and Medical Countermeasure Efficacy

*Major Goals: The overarching goals of the proposed program include validation of the OTE platform for modeling chlorine-mediated pulmonary toxicity, delineating the biochemical toxicity pathways associated with varying levels of chlorine exposure, and screening of compounds, either new or repurposed, as potential countermeasures to pulmonary chlorine injury. Accomplishing these goals would be a significant step towards demonstrating the potential of the OTE platform for the rapid characterization of unknown pulmonary toxins and informing the targeted screening of potential countermeasures.

*Status of Support: Active

Project Number: 75A50119C00058

Name of PD/PI: Atala, Anthony

*Source of Support: DHHS - ASPR BARDA

*Primary Place of Performance: Wake Forest University Health Sciences

Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2019– 06/2022

* Total Award Amount (including Indirect Costs):

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2019	2.40
2. 2020	2.40
3. 2021	2.40
4. 2022	2.40

*Title: Investigation of Non-Invasive Pulsed Electromagnetic Field (PEMF) Therapy for Female Patients with Interstitial Cystitis / Bladder Pain Syndrome (IC/BPS)

*Major Goals: Interstitial cystitis/bladder pain syndrome (IC/BPS) represents a potentially life-long chronic pain disorder that is challenging to diagnose, difficult to treat, and often results in a severely diminished quality-of-life. In this pilot and feasibility clinical trial, we will evaluate the safety and efficacy of a non-invasive therapeutic strategy, pulsed electromagnetic field therapy (PEMF), for pain management in IC/BPS patient subgroups. The goal is to provide preliminary data that will lead to large, hypothesis-driven, multi-center clinical trials.

*Status of Support: Active

Project Number: R01DK12833

Name of PD/PI: Walker, Stephen J.

*Source of Support: NIH

Name of Individual: Walker, Stephen J.
Commons ID: swalker

*Primary Place of Performance: Wake Forest University Health Sciences

Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/2022 – 03/2025

* Total Award Amount (including Indirect Costs):

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	3.60
2. 2023	3.60
3. 2024	3.60
4. 2025	3.60

PENDING

*Title: Prenatal Cell and Gene Therapy for Hemophilia A

*Major Goals: Address critical unanswered biological and efficacy questions regarding IUTx for HA, and to use these data to provide a safer, curative therapy for HA patients.

*Status of Support: Pending

Project Number: R01 PA-20-185 (resubmission

Name of PD/PI: Almeida-Porada, Graca

*Source of Support: NIH

*Primary Place of Performance: Wake Forest University Health Sciences

Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2022 – 06/2026

* Total Award Amount (including Indirect Costs):

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	0.12
2. 2023	0.12
3. 2024	0.12
4. 2025	0.12
5. 2026	0.12

IN-KIND

*Summary of In-Kind Contribution: Human blood, serum, and stool samples provided by Dr. James Adams and Rosa Krajmalnik-Brown at Arizona State University.

*Status of Support: Active

*Primary Place of Performance: Wake Forest University Health Sciences

Project/Proposal Start and End Date (MM/YYYY) (if available): 05/2020 – 04/2023

Name of Individual: Walker, Stephen J.
Commons ID: swalker

*Person Months (Calendar/Academic/Summer) per budget period

Year (YYYY)	Person Months (##.##)
1. [enter year 1]	N/A
2. [enter year 2]	N/A
3. [enter year 3]	N/A
4. [enter year 4]	N/A
5. [enter year 5]	N/A

*Estimated Dollar Value of In-Kind Information: N/A

***Overlap** (summarized for each individual):

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

*Signature: DocuSigned by: Steve Walker B027979A983444A...

Date: 3/25/2022