AWARD NUMBER: W81XWH-17-1-0682

TITLE: Measurement of Biomarkers in Samples Collected in a Coenzyme Q10 Treatment Trial in Gulf War Illness and Control Subjects

PRINCIPAL INVESTIGATOR: Maria Abreu, PhD

CONTRACTING ORGANIZATION:
South Florida VA Foundation for Research and Education Inc.
Miami, FL

REPORT DATE: October 2020

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
October 2020	Annual	30Sep2019-29Sep2020

4. TITLE AND SUBTITLE

Measurement of Biomarkers in Samples Collected in a Coenzyme Q10 Treatment Trial in Gulf War Illness and Control Subjects

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0682

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

The project originally was a partnership of a clinician, Dr. Klimas, and a laboratory scientist, Dr. Fletcher. After Dr. Fletcher's retirement, Dr. Abreu took over the PI role in which the goal is to examine the usefulness of biomarkers in the treatment of GWI, and predicting the subgroup most responsive to an antioxidant intervention, ubiquinol. It is hypothesized that CoQ10 will favorably affect the biomarker signature found in GWI patients. Specifically, we seek to perform biomarker analysis before and after two, four, and six months of therapy with blood collections from subjects in the clinical trial. Laboratory assessments consists of plasma cytokines, natural killer cell function, plasma levels of neuropeptide Y, immune- phenotyping studies using flow cytometry, and mitochondrial function; This data will then be used to correlate these biomarkers with symptom clusters, illness severity and their usefulness in predicting responders to the intervention. Recruitment is closed in the Phase III placebo control treatment trial of CoQ10, which is sponsored by the VA. In this collaborative DoD study, we are assessing the biomarkers (pre and post-treatment) from the plasma, serum, and PBMCs obtained from 100 participants.
As this is a blind, randomized trial, we cannot compare the participants treated with CoQ10 (200mg) to the matched GWI participant placebo group until the end of the study, though we have a growing data set of baseline data for the cohorts. The final subject completed the study drug at the end of October; batched assays are now being completed. We will begin analysis and link biomarker changes with clinical response with our BU colleagues who are managing the VA Merit trial data set. Final analysis and manuscript submission should be complete by Spring 2021.

15. SUBJECT TERMS

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	10	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

Researchers have been investigating the cause and potential treatments of Gulf War Illness for two decades, but to date there have been no successful Phase III trials, and no established treatments beyond palliation of individual symptoms and behavioral aids to cope with chronic illness. The current study examines biomarkers useful as surrogates of severity as well as predictors of response to CoQ10 therapy from biospecimens collected during a current VA Phase III placebo control treatment trial. It is hypothesized that CoQ10 will favorably impact the biomarker signature found in GWI patients and aid in understanding the utility of biomarkers in clinical trials in GWI as well as the role of immune activation, oxidative stress and mitochondrial dysfunction in this illness. We are performing biomarker studies before and after two, four, and six months of therapy, with blood collections from subjects in the clinical trial and laboratory assessments of plasma cytokines, natural killer cell function, plasma neuropeptide Y, cell population studies by flow cytometry, and mitochondrial function. We also are correlating these biomarkers with symptom clusters, illness severity and the usefulness in predicting responders to the intervention. We are assessing the biomarkers (pre and post-treatment) from the plasma, serum, and PBMCs obtained from participants with GWI treated with CoQ10 (200mg) compared to matched participants with GWI placebo group. Our laboratory measures NK cytotoxicity assays, pro-inflammatory and anti-inflammatory using a 18 multiplex Cytokine Array, Flow cytometry to determine lymphocyte subsets and assessment of cell surface proteins, Autonomic nervous systems evaluation including: catecholamine, epinephrine, norepinephrine and NPY measurements, and Mitochondria function including: mitochondrial respiration and glycolysis. Results from these studies will be used to map changes in marker co-expression. Using classical multivariate projection to latent structure, we will identify and compare statistical patterns associating symptoms clusters and interactions within cellular and molecular markers in the blood pre and post-treatment.

2. KEYWORDS:

CoQ10, Gulf War illness, biomarkers, bioenergetics, inflammation, immune function

3. ACCOMPLISHMENTS:

What were the major goals of the project?

	Time-line Months	**Site 1** Initiating PI	**Site 2** Partner PI	**Percent Completed**
Major Task 1 (Specific Aim 1): Perform biomarkers studies before and after (2, 4, and 6 months) therapy with blood and saliva collections and laboratory assessments of oxidative stress and mitochondrial function, CoQ10 levels, cytokines, natural killer cell function, neuropeptide Y, hormone and cell population				

studies.				
Subtask 1: Submit documents for local IRB review at VAMC	1-3		Dr. Klimas	100%
Subtask 2: Monitor and report adverse events to IRB, Data monitoring board chair (*this is a function of VA study, and is up to date 9/18 and 12/18)	12-36		Dr. Klimas	See note*
Subtask 3: Measure plasma cytokines before and after (2, 4, and 6 months) therapy with CoQ10.	12-36	Dr. Abreu		58%
Subtask 4: Determine lymphocyte subsets in PBMC using flow cytometry.	12-36	Dr. Abreu		100%
Subtask 5: Perform NK cytotoxicity assays using PBMC collected.	12-36	Dr. Abreu		100%
Subtask 6: Measure plasma Neuropeptide Y levels using ELISA assays. (batched at end of study)	12-36	Dr. Abreu		5%
Subtask 7: Assess mitochondrial function after treatment with CoQ10 (batched with baseline, 3 mo, 6 mo samples)	12-36	Dr. Abreu		10%
Subtask 8: Perform assays monitoring antioxidant and methylation pathway metabolites levels in addition to measuring catecholamine levels in plasma. (batched with baseline, 3 mo, 6 mo samples)	12-36	Dr. Abreu		50%
Maintaining quality and timely data entry.	6-36	Dr. Abreu	Dr. Klimas	Ongoing
Major Task 2 (Specific Aim 2): Explore whether there is a biomarker or group of biomarkers that predict response to CoQ10 and whether CoQ10 supplementation results in alternations of biomarkers (in collaboration with Dr. Broderick).	8-36	Dr. Abreu	Dr. Klimas	Ongoing
Subtask 1: Numerical analysis of laboratory markers.	12-36	Dr. Abreu	Dr. Klimas	5%
Subtask 2: Mapping changes in marker co-expression. (requires baseline, 3 mo, 6 mo samples)	12-36	Dr. Abreu	Dr. Klimas	5%
Subtask 3: Match biomarkers to specific symptom clusters and illness severity indicators. (requires minimum of 50 subjects to begin preliminary analysis)	12-36	Dr. Abreu	Dr. Klimas	0%
Subtask 4: Correlate these biomarkers, with symptom	12-36	Dr.	Dr.	

clusters, illness and illness severity indicators in terms of statistical significance in order to determine the usefulness in predicting responders to the intervention. (requires minimum of 50 subjects to begin preliminary analysis)		Abreu	Klimas	0%
Subtask 5: Prepare study results in technical terms in tabular format, with response to key hypotheses, in relation to the literature and define next steps for future research. (insufficient data – should have preliminary data set robust enough to do so at next annual)	12-36	Dr. Abreu	Dr. Klimas	0%
Subtask 6: Prepare study results in lay terms for distribution to veteran groups and define next steps for future research. (should have sufficient sample at next annual report to release early findings)	24-36	Dr. Abreu	Dr. Klimas	0%
Milestone: Manuscript on biomarkers and their response to CoQ10 therapy.	24-36	Dr. Abreu	Dr. Klimas	0%

What was accomplished under these goals?

For Specific Aim 1, Dr. Klimas submitted the study documents for local IRB review at the Miami VAMC. Approval was received at the Miami VAMC. For all participant samples collected to date, Drs. Abreu and Klimas are maintaining the quality of the samples and assay preparations and performing data entry in a timely manner. For Specific Aim 2, Drs. Abreu and Klimas are working with Dr. Broderick on exploring whether there is a biomarker or group of biomarkers that may predict response to CoQ10. The question on whether CoQ10 supplementation results in alternations of biomarkers will not be complete until the study cohorts are robust and can be unblinded. The study team will continue to conduct this analysis throughout the remainder of the study based on biomarkers (e.g. change in ubiquinol levels in coded data sets as they relate to symptoms, severity, and other biomarkers).

The Clinical Immunology Laboratory personnel biobanked samples for biomarker studies of samples collected before and after 2, 4, and 6 months of therapy, with blood and saliva collections and laboratory assessments of the participants for the VA CSR&D clinical trial, A Randomized, Double blind, Placebo controlled Phase III Trial of Coenzyme Q10 in Gulf War Illness. After experiencing significant delays in IRB approvals at the 4 sites: recruitment began at all 4 VA sites in January 2018, with the revised goal of recruiting 30 participants by the end of year 1 in January 2019. The sites were challenged to meet the recruitment goals and complete the study recruitment. Recruitment was closed in the Phase III placebo control treatment trial of CoQ10. The final Miami VA participant completed study drug the end of October, batched assays are now being completed. Further analysis will be linked to clinical response with our BU colleagues who are managing the VA Merit trial data set. Final analysis and manuscript submission should be complete by Spring 2021.

What opportunities for training and professional development has the project provided? The laboratory staff have been trained and are proficient in the assays being performed. The Clinical investigators of the VA study are aware and supportive of this study's goals and functioning as clinical collaborators.

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? We will continue to perform assays and analyze all collected biospecimens, according to the protocol.

4. **IMPACT:** Too soon in the study to report results and impact.

What was the impact on the development of the principal discipline(s) of the project? Nothing to Report. Too soon in the study to report results and impact.

What was the impact on other disciplines? Nothing to Report. Too soon in the study to report results and impact.

What was the impact on technology transfer? Nothing to Report. Too soon in the study to report results and impact.

What was the impact on society beyond science and technology? Nothing to Report. Too soon in the study to report results and impact.

5. CHANGES/PROBLEMS: Nothing to Report

Changes in approach and reasons for change Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
The research team did not encounter problems in the laboratory; however, the study was impacted by the slow enrollment in the Phase III clinical trial of CoQ10, a VA funded and VA based study as well as the Coronavirus pandemic. Nevertheless, assays are being performed and there has been increasing productivity completing flow cytometry analysis, Natural Killer cell function, cortisol levels analysis, and cytokine has been completed for all participants that completed every time point of the trial.

Changes that had a significant impact on expenditures
We have deliberately slowed year 1 and 2 spending in this study to reserve sufficient funds in this continuation year to complete the goals of the study. We have sufficient funding to do so.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents Nothing to Report

Significant changes in use of biohazards and/or select agents Nothing to Report

6. PRODUCTS: Nothing to Report

- **Publications, conference papers, and presentations** Nothing to Report

  Journal publications Nothing to Report

  Books or other non-periodical, one-time publications. Nothing to Report

  Other publications, conference papers and presentations. Nothing to Report

- **Website(s) or other Internet site(s)** the study is listed in clincialtrials.gov and has been described in lay media sites.

- **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 the project?

Name:	Maria Abreu, PhD
Project Role:	Initiating PI
Research Identifier:	eCommons: mabreu
Nearest person month	1
Contribution to Project:	Oversees the entire research project. She standardizes protocols, supervises staff, reviews quality control data, interprets data, and assists in manuscript preparation.
Funding Support:	NIH, DoD

Name:	Nancy Klimas, MD
Project Role:	Collaborating/Partnering PI
Research Identifier:	eCommons: nklimas
Nearest person month	1

Contribution to Project:	PI of the parent national clinical trial from where the bio-specimens originate. She is the medical and research director of the Miami VAMC GWI clinical and research program. She works with Dr. Fletcher, supervises staff, and assists in all aspects of the research project.
Funding Support:	NIH, DoD, VA, CDC

Name:	Gordon Broderick, PhD
Project Role:	Co-Investigator
Research Identifier:	eCommons: gbroderick
Nearest person month worked:	3
Contribution to Project:	Works on the computational models for human research and assist in findings.
Funding Support:	NIH, VA

Name:	Kristina Aenlle, PhD
Project Role:	Co-Investigator
Research Identifier:	eCommons: kaenlle
Nearest person month worked:	2
Contribution to Project:	Works with the investigators to analyze the results of assays and laboratory tests to distinguish which biomarkers can explain the effects of CoQ10. In addition to the analysis of the lab tests, communicating with other members of the research team, and writing reports and manuscripts with the other investigators, she investigates the safety and effectiveness of CoQ10 and helps Dr. Klimas to explain the effects based upon the results of the lab tests.
Funding Support:	DoD, VA

Name:	David Freeman
Project Role:	Data Analyst
Research Identifier:	
Nearest person month worked:	2

Contribution to Project:	Under the supervision of Dr. Klimas, with consultation from Dr. Broderick, this individual will apply conventional and high dimensional multivariate statistical techniques to identify biomarkers and biomarker clusters that distinguish illness groups as well as treatment responsive subtypes from non-responsive subtypes.
Funding Support:	DoD

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

W81XWH1820062 (Klimas) 9/30/2018-9/29/2022 DoD GWIRP
The Gulf War Illness Clinical Trials and Interventions Consortium (GWICTIC)

GW170055 (Sullivan) 9/01/2018-8/31/2021 DoD GWIRP
Boston Biorepository, Recruitment and Integrative Network (BBRAIN)

W81XWH1810660 (Nathanson) 9/30/2018-9/29/2021 DoD GWIRP
Immunomodulation in GWI

What other organizations were involved as partners? Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Duplicative report is being submitted for the Collaborating/Partnering to **https://ebrap.org/ for Nancy Klimas, MD.**

QUAD CHARTS:

9. APPENDICES: Nothing to Report.