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

PRINCIPAL INVESTIGATOR: Mary Ann Fletcher, PhD

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

Miami, FL 33125-1624

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The current project is a partnership of a clinician, Dr. Klimas and a laboratory scientist, Dr. Fletcher 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 impact the biomarker signature found in GWI patients. Specifically, we seek to perform 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; and then to correlate these biomarkers with symptom clusters, illness severity and their usefulness in predicting responders to the intervention. Recruitment is ongoing in the Phase III placebo control treatment trial of CoQ10, which Is sponsored by the VA. In this collaborative DoD study, we are4 assessing the biomarkers (pre and post-treatment) from the plasma, serum, and PBMCs obtained from the 27 participants to date. As this is a blind, randomized trial, we cannot compare the participants treated with CoQ10 (200mg) compared to the matched GWI participant placebo group until the end of the study, though we have a growing data set of baseline data or the cohorts. Our timeline is tied to that of the ongoing clinical trial, which just entered the 2nd year of a 3 year timeline. In this first year, we have successfully worked out the logistics of obtaining and processing samples from the 4 sites and linking data to the clinical assessment data set. We have adjusted our anticipated spending to reserve funding for an anticipated continuation year, based on the timeline of the VA based clinical trial.

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	UU Unclassified	11	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 or its symptoms 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: *Provide a brief list of keywords (limit to 20 words).*

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

3. ACCOMPLISHMENTS:

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

	Time-line Months	**Site 1** Initiating PI	**Site 2** Partnering 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. Fletcher		15%
Subtask 4: Determine lymphocyte subsets in PBMC using flow cytometry.	12-36	Dr. Fletcher		15%
Subtask 5: Perform NK cytotoxicity assays using PBMC collected.	12-36	Dr. Fletcher		15%
Subtask 6: Measure plasma Neuropeptide Y levels using ELISA assays. (batched at end of study)	12-36	Dr. Fletcher		0%
Subtask 7: Assess mitochondrial function after treatment with CoQ10 (batched with baseline, 3 mo, 6 mo samples)	12-36	Dr. Fletcher		0%
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. Fletcher		0%
Maintaining quality and timely data entry.	6-36	Dr. Fletcher	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. Fletcher	Dr. Klimas	Ongoing
Subtask 1: Numerical analysis of laboratory markers.	12-36	Dr. Fletcher	Dr. Klimas	5%
Subtask 2: Mapping changes in marker co-expression. (requires baseline, 3 mo, 6 mo samples)	12-36	Dr. Fletcher	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. Fletcher	Dr. Klimas	0%
Subtask 4: Correlate these biomarkers, with symptom 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)	12-36	Dr. Fletcher	Dr. 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. Fletcher	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. Fletcher	Dr. Klimas	0%
Milestone: Manuscript on biomarkers and their response to CoQ10 therapy.	24-36	Dr. Fletcher	Dr. Klimas	0%

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

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. Fletcher 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. Fletcher and Klimas are working with Dr. Broderick and have begun the process of exploring whether there is a biomarker or group of biomarkers that may predict response to CoQ10 and whether CoQ10 supplementation results in alternations of biomarkers will not be complete until the study cohorts are robust and can be unblinded. They 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 continues to biobank 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. As of 11/2018, the total number of participants recruited is 27 participants, the number of participants by VA site is: 9 Miami, 3 Boston, 4 Bronx, and 11 Minneapolis. The sites are on track to meet the recruitment goals of this 3-year study and complete the study by spring of 2021. As a result, this study did not spend funds in the initial year and began the laboratory assessments as the study initiated recruitment this past year (2018). Adjusting the DoD study to match the recruitment and assessment schedule of the VA trial, we are targeted to complete this study with a synchronized timeline.

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 studies 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? *Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

We will continue to process and analyze all collected biospecimens, according to the protocol; We anticipate roughly half of the cohort will be enrolled in the coming year through the VA clinical trial (100 GWI veterans).

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
Describe problems or delays encountered during the reporting period 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. These challenges were due in part to the change of personnel who serve as Coordinators at the 4 sites, entailing additional training in the study assessment procedures. All sites experienced recruitment challenges. At the Minneapolis VA, the research team discovered that the low reimbursement impacted potential participants who travel a far distance for the 5 required VA on-site visits. The study received permission from the VA CSR&D Data Monitoring Committee to increase study compensation.

During the next reporting period, the Phase III clinical trial is implementing multiple strategies to increase the momentum to actively pre-screen, recruit, and enroll participants at all 4 VA sites. The research team is fully staffed and trained at all 4 sites and possesses all of the necessary supplies. The laboratory sample processing are proceeding smoothly.

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 the coming year and the following continuation year to complete the goals of the study. We have sufficient funding to do so.

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

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?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	Mary Ann Fletcher, PhD
Project Role:	Initiating PI
Research Identifier:	eCommons: mfletche
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:	Maria Abreu, PhD
Project Role:	Co-Investigator
Research Identifier:	eCommons: abreumm
Nearest person month worked:	4
Contribution to Project:	Works with the technicians to run the assays on the biospecimens, guides work with specimens stored in the biorepository at Miami VAMC, and ensures quality control of all the laboratory functions as well as collect and analyze the data. She communicates with other members of the team and will write reports and manuscripts with the other investigators.
Funding Support:	DoD, VA

Name:	Kristina Aenlle, PhD
Project Role:	Co-Investigator
Research Identifier:	eCommons: kaenlle
Nearest person month worked:	3
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:	To Be Hired
Project Role:	Laboratory Technician
Research Identifier:	
Nearest person month worked:	0

Contribution to Project:	Under the direction of Drs. Fletcher and Abreu, the laboratory technician performs key laboratory experiments including Natural Killer Cell Functional Analysis, Flow Cytometry, and Cytokine Multiplexing analysis. He or she assists in running all of the laboratory assays while conforming to strict high quality standards. In addition, this technician analyzes datasets for entry and maintenance of the laboratory datasets for the shared collaboration effort.
Funding Support:	DoD

Name:	To Be Hired
Project Role:	Data Analyst
Research Identifier:	
Nearest person month worked:	0
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? Nothing to Report.

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://ers.amedd.army.mil.

QUAD CHARTS:

9. APPENDICES: Nothing to Report.